SALIVARY CORTISOL RESPONSE TO FLIES BY MOOSE CALVES

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ABSTRACT: Young animals are particularly vulnerable to environmental stressors that can impair growth and compromise survival. We used salivary cortisol, a glucocorticosteroid hormone, to measure possible stress response of moose calves in Alaska to the abundance of biting and non-biting flies relative to calf age, time of day, and ambient air temperature. We measured salivary cortisol in 5 captive calves up to 4 times daily on 25 days in June-August with corresponding on-host fly collections. We simultaneously collected 2,618 flies, of which 68% were moose flies (*Haematobosca alcis*), 13% coprophagous flies, 9% mosquitoes (Culicidae), 5% horse and deer flies (Tabanidae), and 2% black flies (Simuliidae). The proportion of moose flies increased steadily, representing nearly all flies by study end. Salivary cortisol levels were minimal and similar (<0.2 $\mu g \cdot dL^{-1}$) from 25 to 89 days of age at ambient temperatures ranging from 13 to 34ºC, and did not increase with relative fly abundance. The lack of cortisol response is consistent with observations of minimal reaction to most flies by moose. The dense and fuzzy characteristics of calf pelage may provide a unique, protective barrier to minimize fly bites and exposure to pathogens sometimes associated with wounds or bites. Although a cortisol response to flies was not detected, vector borne pathogens are predicted to increase in a warming climate and warrant surveillance as part of proactive moose management.

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Moose (*Alces alces*) seldom avoid biting and nuisance flies (Diptera) despite persistent attacks from certain species such as the moose fly (*Haematobosca alcis*) (Benedict and Barboza 2022), a biting fly which completes its life cycle in association with moose (Murie 1934, Burger and Anderson 1974). Even when surrounded by >500 flies, moose appear unbothered, exhibiting minimal flinching or stomping (Burger and Anderson 1974, Lankester and Sein 1986, Lankester and Samuel 2007). Although moose might avoid flies by submerging in wetlands and lakes, this behavior is most associated with foraging, thermoregulation, and predator avoidance (Fraser et al. 1984, Renecker and Hudson 1992). Likewise, moose may seemingly avoid flies in roadside areas, but use of these areas is usually associated with forage and sodium consumption (Belovsky and Jordan 1981, Lankester and Samuel 2007). Flies may impose a physiological cost because bite wounds require energy and nutrients to heal; however, that cost is likely negligible during summer when moose are in positive energy balance and experience rapid growth. However, bite wounds may increase the risk of infection by microbes and parasites that influence maintenance, growth, and survival of moose (Benedict and Barboza 2022,

Benedict et al. 2023). As fly numbers increase in mid-June, several open wounds blanketed with flies may develop on the hind legs of adult moose, likely caused by *Onchocerca* sp. (legworm) transmitted by flies (Lankester and Samuel 2007, Benedict et al. 2023).

The glucocorticosteroid hormones cortisol and corticosterone have been used to assess the effects of environmental stressors on a wide variety of mammals (Cook and Schaefer 2002, Sheriff et al. 2011). When an animal experiences an environmental perturbation of sufficient level, the hypothalamic-pituitary-adrenal axis is activated and glucocorticoids are secreted above basal levels (Sapolsky et al. 2000, Reeder and Kramer 2005, Sheriff et al. 2011). While this response may be rapid and short-term, long-term or chronic stimulation can negatively influence immunity, inflammatory responses, reproduction, and growth (Wingfield et al. 1998, Sheriff et al. 2011). Additionally, the stress response consumes energy, using body stores of fat and glycogen that can be limited seasonally (Busch and Hayward 2009, Sheriff et al. 2011).

The principal goal of this project was to measure the cortisol response in moose calves to abundance and type of biting and non-biting flies. We studied calves as they should be most vulnerable to, and best reflect measurable stress due to their naïve immune system and high requirements for growth and development (Campbell et al. 1977, Åsbakk et al. 2005, Witter et al. 2012). We used salivary cortisol as an indicator of stress because it reflects circulating cortisol in the blood, peaking 20–30 min after onset of a stressor (Sheriff et al. 2011). Recent studies with adult female moose (Thompson et al. 2020a) found that rapid increases in ambient air temperature elevated salivary cortisol levels during the day, and increasing daily heat loads from solar radiation increased fecal corticosterone levels among days. Although Thompson et al. (2020a) did not measure fly abundance and diversity, a causal effect may occur because fly abundance and ambient air temperature are directly related (Burger and Anderson 1974). Therefore, we measured salivary cortisol of calves throughout the summer to assess their response to abundance and diversity of flies relative to ambient air temperature, time of day, and age of calves. In support of our hypothesis that calves are physiologically stressed by flies and warm temperatures, we predicted that salivary cortisol would be related directly to fly abundance and ambient air temperature.

METHODS

Capture and Captive Facility

All procedures for care, handling, and experimentation of animals were approved by the Animal Care and Use Committee, Alaska Department of Fish and Game (ADFG), Division of Wildlife Conservation (IACUC protocol no. 0086-2019-38) and by the Agricultural Animal Care and Use Committee, Texas A&M AgriLife Research (AUP 2019-009A). From May through August 2019, we studied 5 captive moose calves held at the Kenai Moose Research Center (MRC) operated by the ADFG on the Kenai National Wildlife Refuge (60° N, 150° W) where captive moose husbandry has been employed for decades (Hundertmark et al. 2000). In general, calves are bottle-fed with milk replacer and provided forage and pelleted ration through weaning (D. P. Thompson, unpublished data), and trained to tolerate collection of non-invasive samples (e.g., saliva, fecal), enter handling areas, and stand on scales to measure body mass.

The 5 study calves were born 21–24 May 2019 – three captive born at the MRC and two wild orphans (Soldotna and Anchorage, Alaska) collected at 8 and 9 days

old based on behavior and hoof wear (Bragulla 1991). The captive born were removed from the mothers 18-h post-partum after ensuring they had suckled and received colostrum. The calves were raised in a 700 m^2 nursery pen enclosed by a 2.4 m high woven wire fence and further protected by an electric fence to exclude predators in the adjacent mid-seral boreal forest. We provided a 4 m^2 covered shelter within the nursery pen along with feed buckets and ad libitum water. Individuals were identified initially by a colored string around their neck that was subsequently replaced with a colored, expandable VHF collar (Mod-415-3, Telonics, Mesa, Arizona, USA) at 2 weeks of age. Calves were monitored closely for alertness, milk intake, injury, and diarrhea. Handlers minimized noise around the calves while habituating them to human contact. At 21 days of age, calves began walking with handlers into a 0.23 km^2 enclosure containing a wetland, mixed-aged boreal forest, black spruce forest, and open meadow where they foraged 2–3 h daily. At 10 weeks, we walked calves twice daily and progressively started leaving them alone in the larger enclosure for the entire day, returning to the nursery pen for bottle feedings, sample collection, and nighttime security.

Sample Collection

Calves were trained to tolerate collection of saliva and flies using apples, pellets, and bananas as incentives. Saliva collections were scheduled prior to a milk meal, which elicited a salivary response but precluded contamination of the sample with milk. Saliva was collected between the bottom teeth and gums with a synthetic swab (SalivaBio Children's Swab, Salimetrics LLC, Carlsbad, California, USA); the sample was stored frozen within an hour of collection. We collected flies aggregating on the calves with a 0.381 m diameter collapsible net (BioQuip, Rancho Dominquez, California, USA) by swiping it overhead and near the skin surface for 2 min (Lloyd and Dipeolu 1974), subsequently transferring flies to a kill jar with acetone.

We collected saliva at 15:30 hr on 18 and 23 June (Julian days 169 and 174), and at 18:30 hr on 21 June (Julian day 172); saliva and flies were collected at 05:30, 12:30, 15:30, and 18:30 hr on 22 June (Julian day 173). From 6 July to 18 August (Julian days 187–230), we collected saliva from each calf on 3 consecutive days each week. Saliva was collected at 15:30 hr on the first and third day, and saliva and flies were collected at 05:30, 12:30, 15:30, and 18:30 hr on the second day. Ambient air temperature (°C), wind speed ($m \cdot s^{-1}$), and relative humidity (hPa) were measured at collection times with a handheld weather meter (Kestrel 4400 Heat Stress Tracker, Kestrel, Boothwyn, Pennsylvania, USA). Saliva samples were analyzed in duplicate with a cortisol ELISA assay (µg/dL; Salivary Cortisol; Salimetrics LLC, Carlsbad, California, USA) and reported as the mean concentration in each sample (Millspaugh et al. 2002, Thompson et al. 2020a, 2020b).

Flies were identified and counted under a dissection microscope and grouped as follows: biting midges (Ceratopogonidae), mosquitoes (Culicidae), moose flies (*Haematobosca alcis*), coprophagous flies (various families), black flies (Simuliidae), horse and deer flies (Tabanidae), snipe flies (Rhagionidae), and other (Table 1; USDA Veterinary Permit 139420). These groups represented all fly families identified on the calves, and those most likely to bite or potentially harass the calves. To confirm identification, total DNA was extracted from representative individual flies using a method modified from the Gentra Puregene Kit (Gentra Systems, Inc., D-5500A). PCR reactions targeting a 710 base pair barcoding region of the COI gene were performed using the primer set, LCO1490,

Table 1. Flies collected during 2-min sampling periods (n = 33) of 5 captive moose calves at the Kenai Moose Research Center, Alaska, USA, June to August 2019. Flies were grouped by morphological identifiers to calculate a rate (Count) per collection (mean ± SE) and a sum of all counts in the group (Total). Representative specimens were confirmed by genetic sequence using the NCBI GenBank database, and the BLAST search.

Common name	Species ID	Genbank Match	% Match	Count	Total
Moose fly	*Haematobosca alcis*	MF886185.1	99.8	54.30 ± 8.13	1792
Coprophagous Flies				10.70 ± 3.83	353
Black scavenger fly	Sepsidae sp.	Morphological ID			
Dump fly	*Hydrotaea scambus*	MF891571.1	98.1		
Dump fly	*Hydrotaea* sp.	KP049063.1	98.6		
Dung fly	*Scathophaga suilla*	KR440263.1	100.0		
Latrine fly	Fanniidae sp.	Morphological ID			
n/a	*Mesembrina decipiens*	KR618635.1	100.0		
n/a	*Morellia podagrica*	KU496783.1	100.0		
Mosquito	*Aedes* sp.	Morphological ID		6.94 ± 2.26	229
Horse and Deer Flies				4.33 ± 1.50	143
Deer fly	*Chrysops exitans*	JF868977.1	99.8		
Deer fly	*Chrysops frigidus*	KU874617.1	99.8		
Horse fly	*Hybomitra affinis*	HM861001.1	99.8		
Black fly	*Simulium verecundum*	KR682101.1	99.8	1.58 ± 0.38	52
Other Flies				0.79 ± 0.20	26
Dance fly	Hybotidae sp.	HQ551771.1	98.8		
Long-legged fly	*Dolichopus* sp.	KM969513.1	100.0		
n/a	*Pegomya* sp.	MG120915.1	99.9		
n/a	*Thricops diaphanus*	HM412371.1	100.0		
Tiger fly	*Coenosia conforma*	HM883164.1	99.5		
Biting midge	Ceratopogonidae sp.	JN291037.1	98.0	0.64 ± 0.38	21
Snipe fly	*Symphoromyia* sp.	JF868466.1	100.0	0.06 ± 0.04	2

and HC02198 (Folmer et al. 1994). Each reaction contained 2.0 µL of DNA, 0.75 µM of each primer, 12.5 µL Taq-Pro Complete (Thomas Scientific, C788T27), and 9.0 µL of deionized water. The amplification process consisted of the following thermal cycles: one cycle of 3 min at 95 °C, followed by 35 cycles of 1 min at 95°C, 1 cycle of 1.5 min at 45°C, one cycle of 2 min at 72°C, and a final extension step for 5 min at 72°C. PCR products were cleaned using the EXOSAP-IT protocol (ThermoFisher, 78201.1.ML). Each sample was prepared for sequencing using a BigDye Terminator v3.1 Cycle Sequencer Kit and protocol (Applied Biosystems, 4337454). Samples were sequenced in an Applied Biosystems 3500 Genetic Analyzer. Chromatograms produced for each sequence were cleaned and aligned using the program Geneious v. 9.1 (Kearse et al. 2012). Sequences were assigned to species using BLAST search of the NCBI GenBank database, and the percent matches are reported in Table 1 (Ferrar 1974, Hanski and Stähls 1990, Kuchta and Savage 2008, Couri and Salas 2010). Fly counts are expressed on the basis of the number of calves in the group in a 2-min window (flies•calf^{-1}).

Calculations and Statistics

We used mixed-effects regression with individual moose and Julian day as random effects to account for repeated measures of dependent variables (STATA 15.1; StataCorp, College Station, Texas, USA). We used the robust "sandwich estimator" for standard errors to relax assumptions of normal distribution and homogeneity of variances (Rabe-Hesketh and Skrondal 2010). We used a reverse stepwise selection procedure for all mixed models, which removed coefficients that were not significantly different from zero. All statistical significance was set at $P \leq 0.05$.

We examined the effect of time with three metrics: age of the calf as a continuous variable (age_d from 1 to 132 days), age of the calf as a categorical variable (age_w; in 9 weeks from 4 to 13), and the time of day as a categorical variable (hours at four collection times from 05:30 to 18:30 hr). The model for salivary cortisol levels included ambient air temperature (T_a) with calf age (age_week) and time of day (collection) as categorical fixed effects: salivary cortisol = T_a + age_w + collection + ε.

We analyzed counts of flies as groups and as total counts with calf age (age_d) as a fixed effect: count = age_d + ε. We examined the effect of total combined fly groups on salivary cortisol levels with the model: salivary cortisol = flies + ε. We also used principal component analysis to derive two scores (PC 1 and PC 2) that indexed variation in the counts of flies across all 8 groups. Fixed effects for PC 1 and PC 2 were used to examine salivary cortisol levels: salivary cortisol = PC 1 + PC 2 + ε.

RESULTS

A total of 49 saliva samples were collected from each calf beginning at age 25–28 days (stable milk intake) and ending at 86–89 days old when milk meals declined to 2–3 daily. Salivary cortisol concentrations were normally distributed, varied minimally (<0.2 $\mu g \cdot dL^{-1}$), and analyzed without transformation. Salivary cortisol was related to time of day, higher at 05:30 hr than at 15:30 and 18:30 hr (Wald $X^2 = 18.19$, $P = 0.001$; Fig. 1A). Salivary cortisol was not related to calf age (Fig. 1B), fly abundance (see beyond), or ambient temperature ($P = 0.182$) that was consistently cooler at the morning collection (05:30 hr).

Flies were netted around the calves up to four times daily on 9 days in June-August (33 collections, Table 1). Flies were sorted into 8 groups via morphological identification, with representative specimens verified molecularly (Table 1). A total of 2,618 flies (predominantly moose flies) were classified, with moose and coprophagous flies combined representing >80% of total abundance: 68.4% moose flies, 13.5% coprophagous flies, 8.75% mosquitoes, 5.5% horse and deer flies, 2.0% black flies, and <1% other flies, biting midges, and snipe flies (Table 1).

Fly abundance was correlated with and increased linearly with calf age (time) and variation among the 8 fly groups. Only moose fly abundance increased substantially with calf age, representing nearly the entire sample by the last collection; conversely, coprophagous flies declined most over time (Fig. 2A). Principal component 1 accounted for 79.9% of the variation in the fly groups, while principal component 2 accounted for only 12.9% (Fig. 2B). Moose flies, the most abundant, strongly influenced PC 1, whereas coprophagous flies strongly influenced PC 2 (Fig. 2B); mosquitoes, horse flies, and deer flies also influenced PC 2. Variation in the counts of coprophagous flies, horse flies, and deer flies were opposite to the counts of mosquitoes. Variation in the counts of black flies, biting midges, snipe flies, and other flies were not associated with either PC 1 or PC 2.

Salivary cortisol levels were not related to fly abundance (Wald $X^2 = 1.12$, $P > 0.05$). Although the PC 1 and PC 2 scores were

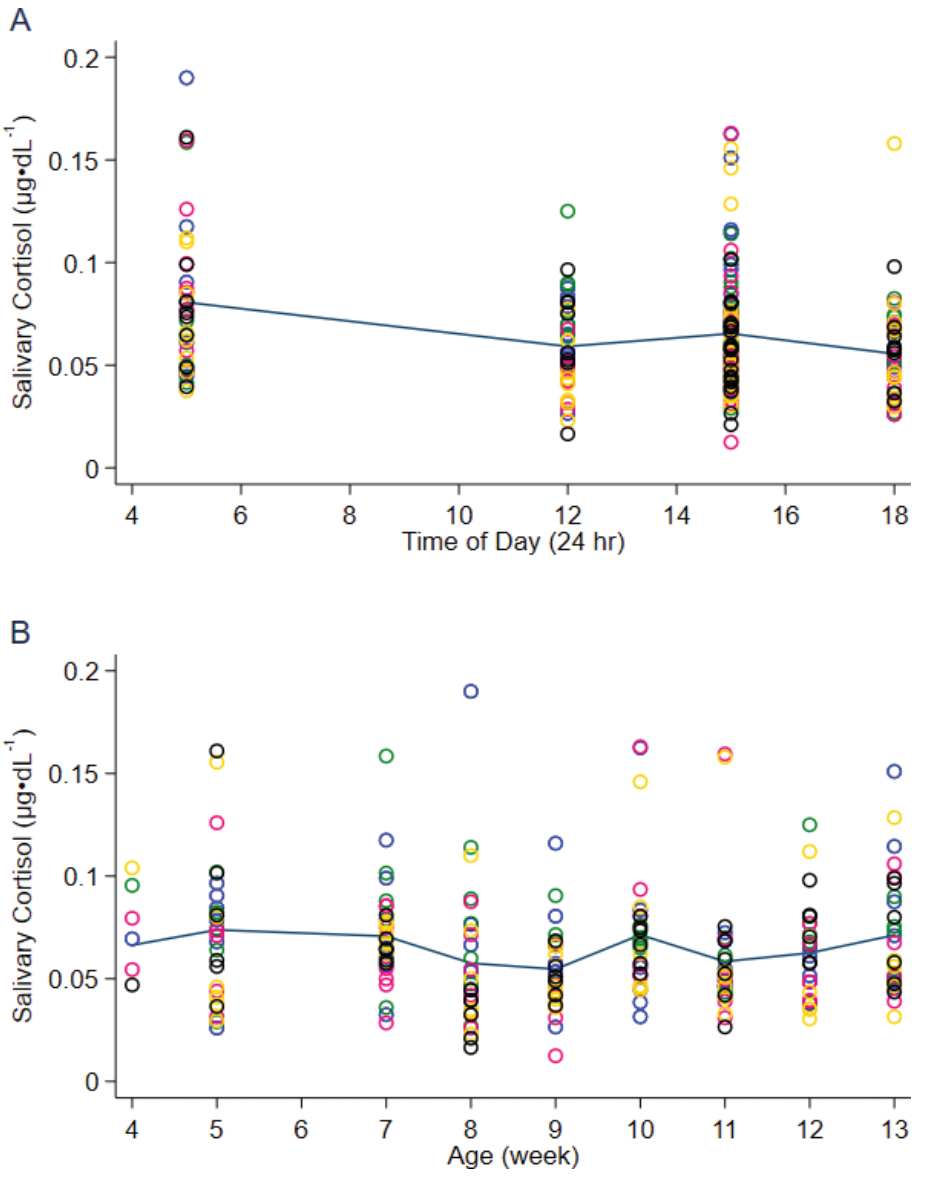

Fig. 1. Marginal predictions and observations (circles), of time of day (A) and calf age (B) on salivary cortisol (µg•dL^{-1}) of moose calves (n = 5 series of colors) based on a mixed model regression with individual and Julian day as random effects to account for repeated measures within individual moose calves at the Kenai Moose Research Center, Alaska, USA, June to August 2019. Model parameters: 241 observations in 5 groups; χ^2 [4 df] = 18.19; P = 0.001. Random effects within individuals were 1.00% of variance.

related statistically to salivary cortisol (Wald X^2 = 59.44, P < 0.001), the absolute change in salivary cortisol was minimal (<0.001 for PC 1, −0.001 for PC 2) over the range of PC scores (Fig. 3).

DISCUSSION

The repeated measures design allowed us to track changes in cortisol response with respect to age (time in days), fly activity/abundance, meals, and ambient temperature. However,

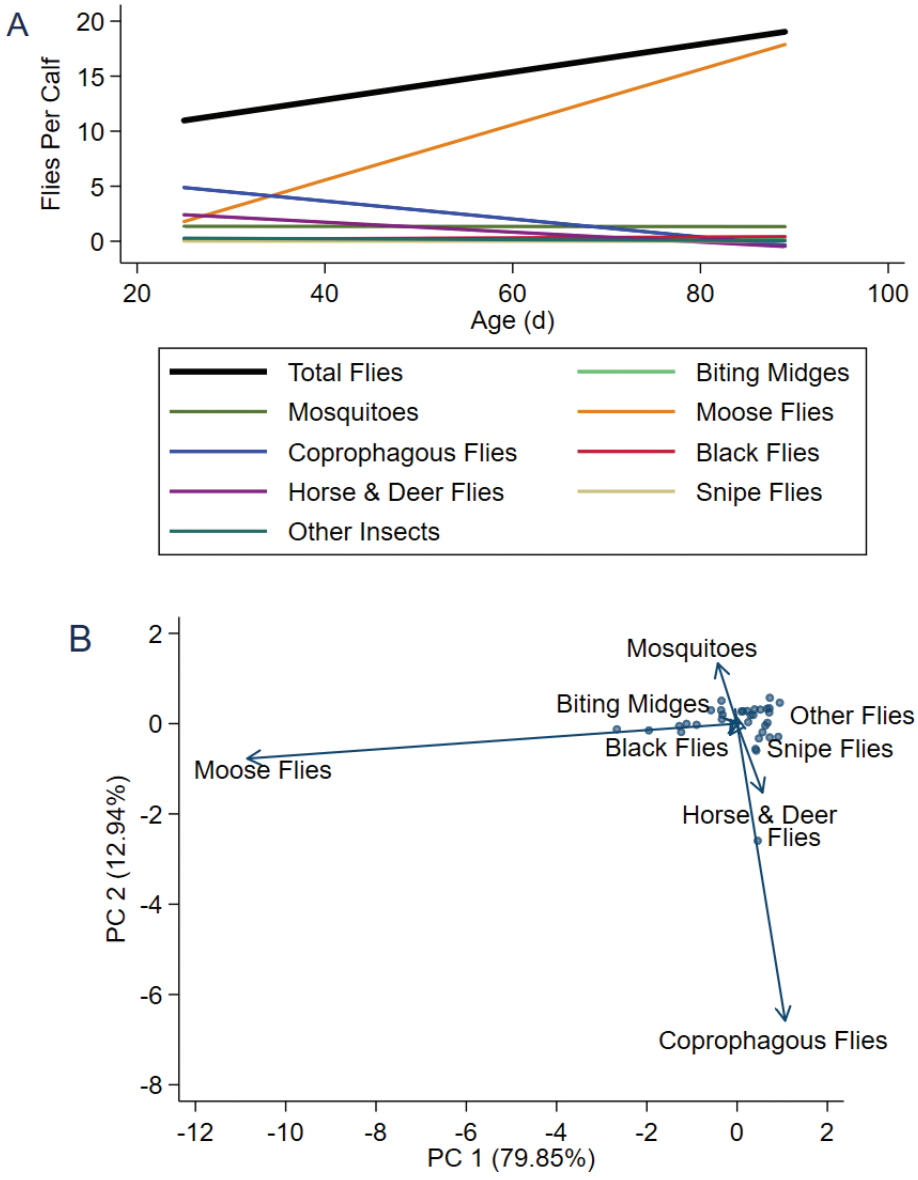

Fig. 2. Plot of marginal predictions (A) of fly count per moose calf in each group of flies against age of calves at the Kenai Moose Research Center, Alaska, USA, June to August 2019. Effects of fly counts on age were all significantly different from zero in mixed model regressions with individual and time (Julian day) as random effects to account for repeated measures. Model parameters: 165 observations in 5 groups; total flies χ^2 [1 df] = 9.38; P = 0.002, biting midges χ^2 [1 df] = 0.97; P = 0.325, mosquitoes χ^2 [1 df] = 0.000; P = 0.974, moose flies χ^2 [1 df] = 53.12; P < 0.001, coprophagous flies χ^2 [1 df] = 31.25; P <0.001, black flies χ^2 [1 df] = 4.40; P =0.036, horse and deer flies χ^2 [1 df] = 66.08; P < 0.001, snipe flies χ^2 [1 df] = 0.72; P = 0.397, other flies χ^2 [1 df] = 19.78; P < 0.001. Random effects within individuals were <0.001% of variance for all models. Principal component analysis (B) of fly counts in 33 collections around moose calves during the summer. Arrows indicate vectors for each fly group in the first and second orthogonal components. Filled circles indicate observations.

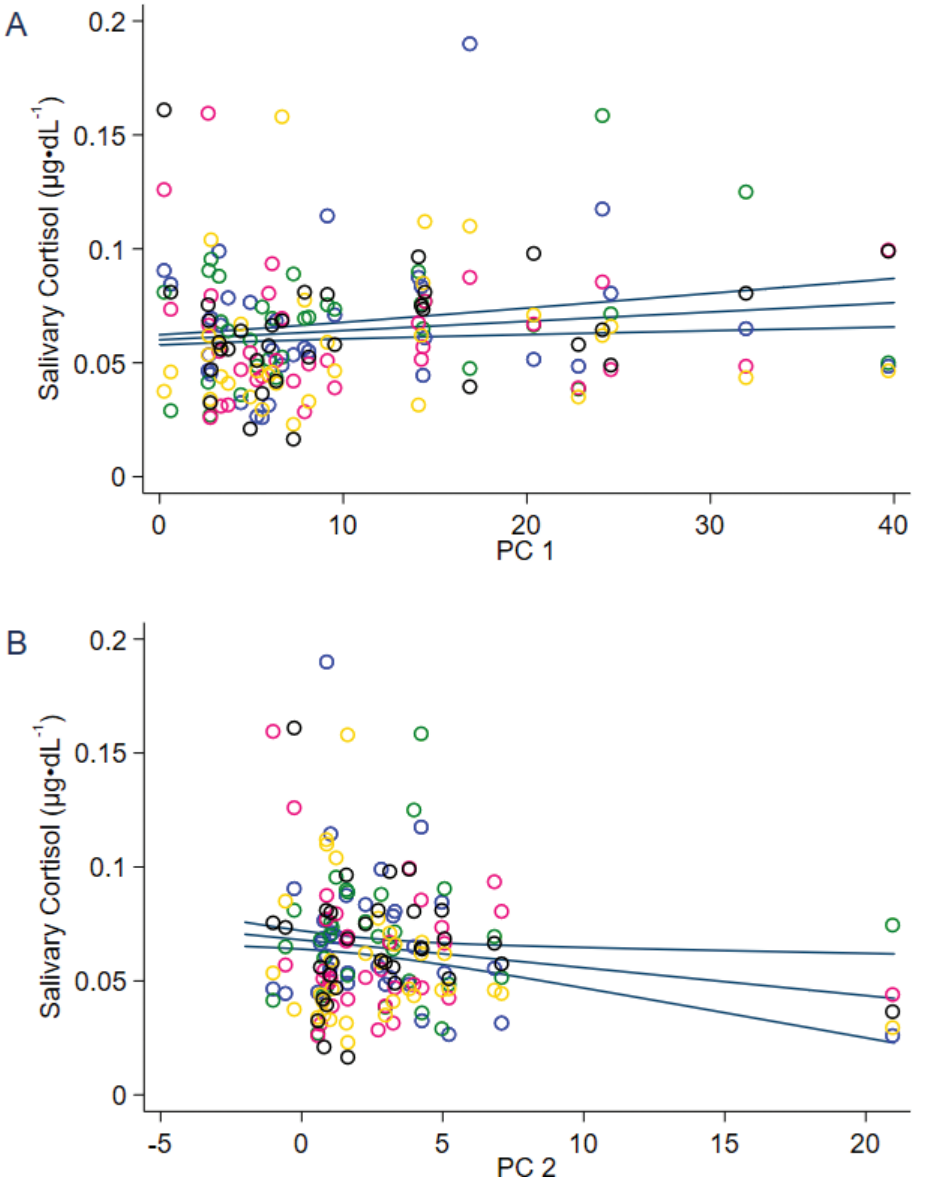

Fig. 3. Relationship between salivary cortisol (µg•dL^{-1}) and two indices of flies on moose calves: PC 1 (A) and PC 2 (B). Indices were derived from principal component analysis of counts of 33 collections of flies around calves (n = 5 series of colors). Lines are predictions with 95% confidence intervals from mixed model regression with individual and Julian day as random effects to account for repeated measures within individual moose calves at the Kenai Moose Research Center, Alaska, USA, June to August 2019. Model parameters: 165 observations in 5 groups; χ^2 [2 df] = 33.68; $P < 0.001$. Random effects within individuals were <0.001% of variance.

the range of salivary cortisol measured in calves (0–0.2 µg·dL^{-1}; Fig. 1) was minimal throughout the study and much lower than that measured in adult female moose (0–3.0 µg·dL^{-1}) (Thompson et al. 2020a). We found small transient changes in salivary cortisol with time of day (highest at early morning feeding), but not with calf age or fly

abundance, even as fly abundance increased steadily over time (Fig. 1B, 2A). Although we found an increase in salivary cortisol with PC1 (Fig. 3A), the predicted rise in concentration (0.007 $\mu g \cdot dl^{-1}$) was less than the sensitivity of the assay (0.012 $\mu g \cdot dL^{-1}$) (Salimetrics LLC, Carlsbad, California, USA). The same assay identified a correlation between change in air temperature and salivary cortisol in adult female moose, with the majority of values in the range of 0–0.5 $\mu g \cdot dL^{-1}$ (Thompson et al. 2020a).

Our study with calves was unique and without comparative studies, and we expected a response in salivary cortisol to fly abundance based on the sensitivity of the assay with adult female moose. The consistent, low levels of salivary cortisol suggest that these hand-reared calves accepted handling and sample collections without stress. Similarly, a study with maternally raised musk deer (*Moschus berezovskii*) found stable cortisol values from birth to weaning (Li et al. 2021). Although maternal stress response during pregnancy may influence that of the offspring in utero and during lactation as in fallow deer fawns (*Dama dama*) (Amin et al. 2021), the levels of salivary cortisol in calves with their mothers is unknown; however, the growth and development of our hand-reared calves were similar to those of maternally raised moose (J. A. Crouse, unpublished data).

Unlike adult moose (Lankester and Samuel 2007; Benedict et al. 2023), calves do not acquire large sores on their hind legs, apparently relying on morphological barriers to largely resist flies. Most flies were collected at the hind end near the tail, with moose flies particularly abundant in this area. Similar observations occur with adult moose, but this area is notably difficult for adults to physically reach and disturb flies (Lankester and Samuel 2007). The dense, fuzzy coat of moose calves may offer a unique protective barrier compared to the summer coat of adults, particularly against non-burrowing species of flies (e.g., moose flies and mosquitoes), effectively shielding the majority of their body except the exposed anus and eyes (Samuel et al. 1986). We observed that damaged areas of the coat were swarmed and bitten by flies.

Flies did not trigger the release of glucocorticoid hormones as a physiological response in moose calves, even though flies may still be perceived as noxious (McEwen and Wingfield 2003, Busch and Hayward 2009). The calves did exhibit signs of annoyance (running, jumping, shaking the head, stomping, twitching) in response to some larger flies (horse and deer flies), even though a release of glucocorticoid hormone was not measured. Considering that moose flies were the most abundant fly at ~35 days and increased in relative abundance thereafter, calves may simply tolerate and become habituated to their presence; varied responses to specific flies is unsurprising given that mouth morphology influences wounding (pain) (Benedict and Barboza 2022). A tipping point or threshold of cortisol response might eventually occur in calves, although we did not identify such through 85+ days. However, a threshold response by moose to deer keds (*Lipoptena cervi*) occurred after prolonged (versus low) exposure to keds triggered a release of glucocorticoids (Madslien et al. 2020).

The higher cortisol values in the morning (05:30 hr, Fig. 1A) coincided with the first milk feeding of the day after calves were alone overnight (Carbonaro et al. 1992a, b); cortisol values were similar at all other feeding times. This small increase at the first daily feeding was unlikely related to stress, and possibly reflected the extended overnight between feedings and "excitement" of the first meal, but more likely, a circadian rhythm in cortisol (Ingram et al.

1999). Regardless, neither fly abundance or ambient air temperature was related to or influenced salivary cortisol values which were essentially unchanged.

Although moose calves appeared resistant to fly harassment and bites based on lack of cortisol response, calves on the Kenai Peninsula are occasionally affected behaviorally (e.g., signs of annoyance to horse and deer flies) and physiologically by certain biting flies. For example, elevated levels of the filarial nematode *Setaria yehi* have been documented in wild and captive calves on the Kenai Peninsula during winter, with morbidity and mortality from peritonitis associated with *S. yehi* migrating from blood vessels in the peritoneum (D. P. Thompson, unpublished data). In Finland, increased fly density during warmer summers was associated with increased peritonitis in reindeer from filarial nematodes (Laaksonen et al. 2007, 2009b). Extended periods of warm temperatures may increase exposure of vulnerable hosts by allowing multiple versus a single life cycle typical of filarial nematodes (Laaksonen et al. 2009b).

The lack of cortisol response in moose calves to fly harassment should not minimize the overall concern associated with increased exposure of moose to flies and other parasites as a warming climate expands the range, abundance, and seasonal activity of all (Kutz et al. 2012, Mallory and Boyce 2018). High moose density could potentially increase the prevalence and deleterious effects of flies and parasites that may reduce productivity and recruitment in moose as documented with winter tick (*Dermacentor albipictus*) parasitism in northeastern North America (Jones et al. 2017). Such effects are important considerations in harvest management (Brown 2011, Rempel 2011) and warrant continued study and monitoring of relationships among moose, disease, and parasites in a warming environment.

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REFERENCES

Amin, B., D. J. Jennings, A. F. Smith, M. Quinn, S. Chari, A. Haigh, D. Matas, L. Koren, and S. Ciuti. 2021. In utero accumulated steroids predict neonate anti-predator response in a wild mammal. Functional Ecology 35: 1255–1267. doi: 10.1111/1365-2435.13790

Åsbakk, K., A. Oksanen, M. Nieminen, R. E. Haugerud, and A. C. Nilssen. 2005. Dynamics of antibodies against hypodermin C in reindeer infested with the reindeer warble fly, *Hypoderma tarandi*. Veterinary Parasitology 129: 323–332. doi: 10.1016/j.vetpar.2005.02.007

Belovsky, G. E., and P. A. Jordan. 1981. Sodium dynamics and adaptations of a moose population. Journal of Mammalogy 62: 613–621. doi: 10.2307/1380408

Benedict, B. M., and P. S. Barboza. 2022. Adverse effects of Diptera flies on northern ungulates: *Rangifer*, *Alces*, and *Bison*. Mammal Review 52: 425–437. doi: 10.1111/mam.12287

_____, _____, J. A. Crouse, K. R. Groch, M. R. Kulpa, D. P. Thompson, G. G. Verocai, and D. J. Wiener. 2023. Sores

of boreal moose reveal a previously unknown genetic lineage of parasitic nematode within the genus *Onchocerca*. PLoS One 18: e0278886. doi: 10.1371/journal.pone.0278886

Bragulla, H. 1991. The deciduous hoof capsule (*Capsula ungulae decidua*) of the equine fetus and newborn foal. Anatomia, Histologia, Embryologia 20: 66–74. doi: 10.1111/j.1439-0264.1991.tb00293.x

Brown, G. S. 2011. Patterns and causes of demographic variation in a harvested moose population: evidence for the effects of climate and density-dependent drivers. Journal of Animal Ecology 80: 1288–1298. doi: 10.1111/j.1365-2656.2011.01875.x

Burger, J. F., and J. R. Anderson. 1974. Taxonomy and life history of the moose fly, *Haematobosca alcis*, and its association with the moose, *Alces alces shirasi* in Yellowstone National Park. Annals of the Entomological Society of America 67: 204–214. doi: 10.1093/aesa/67.2.204

Busch, D. S., and L. S. Hayward. 2009. Stress in a conservation context: a discussion of glucocorticoid actions and how levels change with conservation-relevant variables. Biological Conservation 142: 2844–2853. doi: 10.1016/j.biocon.2009.08.013

Campbell, J., R. White, J. Wright, R. Crookshank, and D. Clanto. 1977. Effects of stable flies on weight gains and feed efficiency of calves on growing or finishing rations. Journal of Economic Entomology 70: 592–594. doi: 10.1093/jee/70.5.592

Carbonaro, D., T. Friend, G. Dellmeier, and L. Nuti. 1992a. Behavioral and physiological responses of dairy goats to food thwarting. Physiology and Behavior 51: 303–308. doi: 10.1016/0031-9384(92)90145-R

_____, _____, _____, and _____. 1992b. Behavioral and physiological responses of dairy goats to isolation. Physiology and Behavior 51: 297-301.

Cook, N., and A. Schaefer. 2002. Stress responses of wapiti (*Cervus elaphus canadensis*) to removal of velvet antler. Canadian Journal of Animal Science 82: 11–17. doi: 10.4141/A01-031

Couri, M. S., and C. Salas. 2010. First record of *Coenosia attenuata* Stein (Diptera, Muscidae) from Chile, with biological notes. Revista Brasileira de Entomologia 54: 144–145. doi: 10.1590/S0085-56262010000100020

Ferrar, P. 1974. *Morellia hortensia* (Wiedemann), a new livestock fly in Australia. Australian Journal of Entomology 13: 71–77. doi: 10.1111/j.1440-6055.1974.tb02293.x

Folmer, O., M. Black, W. Hoeh, R. Lutz, and R. Vrijenhoek. 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Molecular Marine Biology and Biotechnology 3: 294–299.

Fraser, D., E. Chavez, and J. Palohelmo. 1984. Aquatic feeding by moose: selection of plant species and feeding areas in relation to plant chemical composition and characteristics of lakes. Canadian Journal of Zoology 62: 80–87. doi: 10.1139/z84-014

Hanski, I., and G. Stähls. 1990. Prolonged diapause in fungivorous Pegomya flies. Ecological Entomology 15: 241–244. doi: 10.1111/j.1365-2311.1990.tb00805.x

Hundertmark, K. J., T. R. Stephenson, and C. C. Schwartz. 2000. Evaluation and Testing Techniques for Ungulate Management. Alaska Department of Fish and Game, Division of Wildlife Conservation Federal Aid Research Final Performance Report 1 July 1992–30 June 2000, Federal Aid in Wildlife Restoration Study 1.45, Juneau, Alaska, USA.

Ingram, J., J. Crockford, and L. Matthews. 1999. Ultradian, circadian and seasonal rhythms in cortisol secretion and adrenal responsiveness to ACTH and yarding in

unrestrained red deer (*Cervus elaphus*) stags. Journal of Endocrinology 162: 289–300. doi: 10.1677/joe.0.1620289

Jones, H., P. J. Pekins, L. E. Kantar, M. O'Neil, and D. Ellingwood. 2017. Fecundity and summer calf survival of moose during 3 successive years of winter tick epizootics. Alces 53: 85–98.

Kearse, M., R. Moir, A. Wilson, S. Stones-Havas, M. Cheung, S. Sturrock, S. Buxton, A. Cooper, S. Markowitz, and C. Duran. 2012. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics 28: 1647–1649. doi: 10.1093/bioinformatics/bts199

Kuchta, J. S., and J. Savage. 2008. Revision of the New World fauna of *Mesembrina Meigen* (Diptera: Muscidae) with the description of a new Neotropical species. Zootaxa 1689: 29–50. doi: 10.11646/zootaxa.1689.1.2

Kutz, S. J., J. Ducrocq, G. G. Verocai, B. M. Hoar, D. D. Colwell, K. B. Beckmen, L. Polley, B. T. Elkin, and E. P. Hoberg. 2012. Parasites in ungulates of arctic North America and Greenland: a view of contemporary diversity, ecology, and impact in a world under change. Pages 99–252 *in* D. Rollinson and S. I. Hay, editors. Advances in Parasitology. Elsevier, Oxford, United Kingdom.

Laaksonen, S., J. Kuusela, S. Nikander, M. Nylund, and A. Oksanen. 2007. Outbreak of parasitic peritonitis in reindeer in Finland. Veterinary Record 160: 835–841. doi: 10.1136/vr.160.24.835

_____, M. Solismaa, T. Orro, J. Kuusela, S. Saari, R. Kortet, S. Nikander, A. Oksanen, and A. Sukura. 2009. *Setaria tundra* microfilariae in reindeer and other cervids in Finland. Parasitology Research 104: 257. doi: 10.1007/s00436-008-1184-0

Lankester, M., and W. Samuel. 2007. Pests, Parasites and Disease. Pages 479–518 *in* A. W. Franzmann and C. C. Schwartz, editors. Ecology and Management of North American Moose. University Press of Colorado, Boulder, Colorado, USA.

_____, and R. D. Sein. 1986. The moose fly, *Haematobosca alcis* (Muscidae) and skin lesions on moose. Alces 22: 361–375.

Li, Y., T. Zhang, M. Shi, B. Zhang, X. Hu, S. Xu, J. Ding, S. Liu, D. Hu, and D. Rubenstein. 2021. Characterization of intestinal microbiota and fecal cortisol, T3, and IgA in forest musk deer (*Moschus berezovskii*) from birth to weaning. Integrative Zoology 16: 300–312. doi: 10.1111/1749-4877.12522

Lloyd, D., and O. Dipeolu. 1974. Seasonal prevalence of flies feeding on cattle in Northern Nigeria. Tropical Animal Health and Production 6: 231–236. doi: 10.1007/BF02383282

Madslien, K., S. M. Stubsjøen, H. Viljugrein, B. Ytrehus, E. J. Solberg, L. Kapronczai, A. Mysterud, J. Godfroid, D. M. Janz, and M. Cattet. 2020. Hair cortisol concentration and body mass in moose (*Alces alces*) infested with deer keds (*Lipoptena cervi*). Journal of Wildlife Diseases 56: 687–692. doi: 10.7589/2019-07-185

Mallory, C. D., and M. S. Boyce. 2018. Observed and predicted effects of climate change on Arctic caribou and reindeer. Environmental Reviews 26: 13–25. doi: 10.1139/er-2017-0032

McEwen, B. S., and J. C. Wingfield. 2003. The concept of allostasis in biology and biomedicine. Hormones and Behavior 43: 2–15. doi: 10.1016/S0018-506X(02)00024-7

Millspaugh, J. J., B. E. Washburn, M. A. Milanick, J. Beringer, L. P. Hansen, and T. M. Meyer. 2002. Non-invasive techniques for stress assessment in white-tailed deer. Wildlife Society Bulletin 30: 899–907.

Murie, A. 1934. The Moose of Isle Royale. Miscellaneous Publications 25.

University of Michigan Press, Museum of Zoology, University of Michigan, Ann Arbor, Michigan, USA.

Rabe-Hesketh, S., and A. Skrondal. 2010. Multilevel and Longitudinal Modeling Using Stata. Third edition. Stata Press, College Station, Texas, USA.

Reeder, D. M., and K. M. Kramer. 2005. Stress in free-ranging mammals: integrating physiology, ecology, and natural history. Journal of Mammalogy 86: 225–235. doi: 10.1644/BHE-003.1

Rempel, R. S. 2011. Effects of climate change on moose populations: exploring the response horizon through biometric and systems models. Ecological Modelling 222: 3355–3365. doi: 10.1016/j.ecolmodel.2011.07.012

Renecker, L. A., and R. J. Hudson. 1992. Habitat and forage selection of moose in the aspen-dominated boreal forest, central Alberta. Alces 28: 189–201.

Samuel, W., D. Welch, and M. L. Drew. 1986. Shedding of the juvenile and winter hair coars of moose (*Alces alces*) with emphasis on the influence of the winter tick, *Dermacentor albipictus*. Alces 22: 345–360.

Sapolsky, R. M., L. M. Romero, and A. U. Munck. 2000. How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. Endocrine Reviews 21: 55–89. doi: 10.1210/edrv.21.1.0389

Sheriff, M. J., B. Dantzer, B. Delehanty, R. Palme, and R. Boonstra. 2011. Measuring stress in wildlife: techniques for quantifying glucocorticoids. Oecologia 166: 869–887. doi: 10.1007/s00442-011-1943-y

Thompson, D. P., J. A. Crouse, S. Jaques, and P. S. Barboza. 2020a. Redefining physiological responses of moose (*Alces alces*) to warm environmental conditions. Journal of Thermal Biology 90: 102581. doi: 10.1016/j.jtherbio.2020.102581

_____, _____, T. J. McDonough, P. S. Barboza, and S. Jaques. 2020b. Acute thermal and stress response in moose to chemical immobilization. The Journal of Wildlife Management 84: 1051–1062. doi: 10.1002/jwmg.21871

Wingfield, J. C., D. L. Maney, C. W. Breuner, J. D. Jacobs, S. Lynn, M. Ramenofsky, and R. D. Richardson. 1998. Ecological bases of hormone – behavior interactions: the "emergency life history stage". American Zoologist 38: 191–206. doi: 10.1093/icb/38.1.191

Witter, L. A., C. J. Johnson, B. Croft, A. Gunn, and L. M. Poirier. 2012. Gauging climate change effects at local scales: weather-based indices to monitor insect harassment in caribou. Ecological Applications 22: 1838–1851. doi: 10.1890/11-0569.1