



Influence du stress de prédation sur la reproduction de la chèvre de montagne

Mémoire

Frédéric Dulude-De Broin

Maîtrise en biologie - avec mémoire
Maître ès sciences (M. Sc.)

Québec, Canada

INFLUENCE DU STRESS DE PRÉDATION SUR LA REPRODUCTION DE LA CHÈVRE DE MONTAGNE

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Frédéric Dulude-de Broin

Sous la direction de :

Steeve D. Côté, directeur de recherche

Résumé

L'influence de la prédation sur une proie s'étend au-delà de la mortalité directe associée à la consommation d'individus. Dans certains cas, la simple présence d'un prédateur au sein d'un écosystème peut entraîner une réduction drastique de la reproduction des proies. L'induction d'un état de stress physiologique chronique pourrait être le mécanisme à l'origine de cet effet. L'objectif de mon projet était d'évaluer l'influence du stress de prédation sur la reproduction de la chèvre de montagne (*Oreamnos americanus*). Nous avons d'abord validé en captivité une méthode permettant d'évaluer le stress en mesurant la concentration en glucocorticoïdes dans des échantillons de fèces et de poils, puis nous avons analysé une base de données longitudinale sur une population de chèvres de montagne marquées et suivies durant toute leur vie en milieu naturel. Nos résultats indiquent que la prédation peut agir sur la reproduction de la chèvre de montagne par l'induction d'un état de stress chronique. En plus de contribuer à la compréhension des effets indirects de la prédation chez les mammifères, cette étude permet de préciser les causes du déclin d'une population sauvage de chèvres de montagne.

Abstract

The impact of predation on prey goes beyond the direct consumption of individuals. In some cases, the mere presence of predators in an ecosystem can drastically impair prey reproduction. The induction of chronic physiological stress could act as the mechanism underlying such effects. My objective was to assess whether predator-induced stress could impair reproduction in an alpine ungulate, the mountain goat. We first validated a method to measure physiological stress using faeces and hair as biomarkers, and then analysed a long-term data base on a population of wild marked mountain goats. Our results indicate that predation may act on reproduction in mountain goats through the induction of chronic stress. In addition to its contribution to the understanding of indirect effects of predation in mammals, this study clarifies the causes behind the decline of a wild mountain goat population.

Table des matières

Résumé.....	ii
Abstract	iii
Table des matières	iv
Liste des figures.....	vii
Liste des tableaux	xii
Liste des abréviations	xii
Épigraphie	xiii
Remerciements	xv
Avant-propos	xviii
Introduction.....	1
Les effets directs et indirects de la préation	1
Le mécanisme incertain des effets indirects de la préation	2
L'homéostasie, l'allostasie et le stress	3
L'hypothèse préation-stress.....	5
La chèvre de montagne, un ongulé alpin comme modèle d'étude	6
La population de Caw Ridge	7
Note sur la mesure du stress en milieu naturel.....	8
Objectifs	9
1. Faecal metabolites and Hair cortisol as biological markers of HPA-axis activity in the Rocky Mountain Goat.....	10
1.1 Résumé.....	11
1.2 Abstract.....	12
1.3 Introduction	13
1.4 Materials and methods	15
1.4.1 Animals.....	15
1.4.2 Faecal ACTH challenge	15
1.4.3 Hair ACTH challenge.....	17
1.4.4 Age, sex, reproductive status, date and hair type differences	17
1.4.5 FGM degradation experiment.....	18
1.4.6 Glucocorticoid extraction	18
1.4.7 HPLC Analysis	19
1.4.8 Immunoassay	20

1.4.9 Statistical analysis.....	20
1.5 Results.....	22
1.5.1 Validity of immunoassay.....	22
1.5.2 ACTH challenge	24
1.5.3 Faecal sample collection date and hair type.....	25
1.5.4 Age, sex and reproductive status	27
1.5.5 Faecal metabolites degradation.....	29
1.6 Discussion.....	30
1.6.1 Validity of immunoassay.....	30
1.6.2 ACTH challenge	31
1.6.3 Faecal sample collection date and hair type.....	32
1.6.4 Age, sex and reproductive status	33
1.6.5 Faecal metabolites degradation.....	35
1.7 Acknowledgements.....	36
1.8 Supplementary material	37
1.8.1 ACTH challenge timetable.....	37
1.8.2 AIC table for the effect of faecal sample collection date.....	38
1.8.3 Grouping patterns for the effect of age class	39
1.8.4 Biochemical validations	40
2. Predation risk and mountain goat reproduction: Evidence for stress-induced breeding suppression in a wild ungulate.....	42
2.1 Résumé.....	43
2.2 Abstract.....	44
2.3 Introduction	45
2.4 Materials and Methods.....	48
2.4.1 Study area and population.....	48
2.4.2 Captures and body mass measurements	49
2.4.3 Reproductive success and demographic data.....	49
2.4.4 Predation risk	50
2.4.5 Glucocorticoid measurements.....	51
2.4.6 Resource availability	52
2.4.7 Statistical analysis.....	53
2.5 Results.....	55

2.6 Discussion.....	59
2.7 Acknowledgements.....	64
2.8 Supplementary material	65
2.8.1 Age-specific mass.....	65
2.8.2 Influence of freezing delay on faecal glucocorticoid metabolites	67
2.8.3 Adjustment for seasonal variation in basal glucocorticoid concentration.....	68
2.8.4 Predictive models for annual FGM and HCC estimates	70
2.8.5 Path analyses based on a data set limited to samples from females	72
2.8.6 Path analyses at the individual level.....	74
2.8.7 Values used for standardizing estimates	76
Conclusion	77
Validation des fèces et des poils comme marqueurs du stress physiologique.....	77
Influence du stress de préation sur la reproduction.....	78
Le déclin de la population de Caw Ridge	80
Limites de l'étude.....	81
Perspectives futures.....	83
Conclusion générale	85
Bibliographie	87

Liste des figures

Figure 0.1 Réactions neuroendocrines des deux axes de réponse au stress suite à la détection d'un facteur de stress. L'axe sympatho-surrenalien (SAM) stimule la production de catécholamines par les glandes surrénales, alors que l'axe hypothalamo-hypophysosurrénalien (HPA) entraîne la production de glucocorticoïdes via la libération en cascade de corticolibérine (CRH) et d'adrénocorticotrophine (ACTH).

Figure 1.1 Reverse phase high performance liquid chromatography (HPLC) separation of pooled faecal extracts from captive mountain goats: (A) chromatogram and (B) immunoreactivity profile as determined by cortisol and corticosterone enzyme immunoassay.

Figure 1.2 Faecal glucocorticoid metabolite (FGM) concentrations of two adult females (diamond and triangle) and one adult male (circle) mountain goat following one adrenocortotropic hormone (ACTH) injection. Black squares are baseline group composite samples. Shaded grey area represents 95% confidence interval of the average baseline concentration.

Figure 1.3 Hair cortisol concentrations (HCC) of two adult females (diamond and triangle), one yearling female (asterisk) and one adult male (circle) mountain goat before and after a trial of 5 repeated adrenocortotropic hormone (ACTH) injections.

Figure 1.4 Faecal glucocorticoid metabolite (FGM) concentration during summer in mountain goats from the Caw Ridge population (Alberta) sampled between 2000 and 2016. Dot size is proportional to sample size. The fitted quadratic regression is presented with its 95% confidence interval and includes age class and sex as fixed covariates and goat identity and year as random factors.

Figure 1.5 Correlation between hair cortisol concentrations (HCC) of guard and undercoat hair in mountain goats, at Caw Ridge, Alberta (2000-2016) from 50 samples that contained both hair types. Linear regression is presented with 95% confidence interval and was fitted without the influential point represented by an empty circle (Cook's distance 9.17).

Figure 1.6 Average hair cortisol concentrations according to age-classes in mountain goats, at Caw Ridge, Alberta (2000-2016). Error bars represent 95% confidence intervals, letters indicate grouping structure determined by multiple comparisons.

Figure 1.7 Variation of FGM concentration in mountain goats, at Caw Ridge, Alberta (2000-2016), after 0, 4, 8 and 21 hours of exposure to environmental conditions either in the sun (square) or in a bag (circle). Error bars represent 95% confidence intervals. Ambient shade temperature recorded at 0, 4, 8 and 21 hours is shown above graph.

Figure 1.S1 Schedule for the faecal ACTH challenge conducted on two adult females and one adult male mountain goat at the Calgary zoo from 27 November to 5 December 2017.

Figure 1.S2 Recovery of exogenous cortisol in pooled a) faecal and b) hair extracts from mountain goats, at Caw Ridge, Alberta (2000-2016).

Figure 1.S3 Comparison of serial dilutions of 5 pooled a) faecal and b) hair extracts (squares) from mountain goats, at Caw Ridge, Alberta (2000-2016) to a cortisol standard curve (diamonds) to assess parallelism.

Figure 2.1 The hypothesized causal model linking predation risk to female reproduction in mountain goats. High predation risk is expected to reduce fertility through the induction of chronic physiological stress. Predation risk could also impact reproduction through alternative mechanisms (e.g. trade-off between food and safety). Among environmental covariates, resources availability could impact stress and population size could impact stress and reproduction. Solid arrows represent the predicted relationships based on the predation-stress hypothesis, dashed arrows represent other potential relationships.

Figure 2.2 Annual variation in demography and predator occurrence at Caw Ridge, Alberta (1989-2017). **A** Total population size on 1 June; **B** Proportion of adult females (≥ 3 years) that gave birth; **C** Number of predator sightings per 100 days of field work (recorded only from 1994); **D** Age-specific mass and 95% CI of adult females (in kg; residuals of mass when accounting for age and seasonal mass variations), where positive values represent years when adult females were heavier than other years during the study period.

Figure 2.3 Annual faecal glucocorticoid metabolites concentration (FGM) in relation with annual hair cortisol concentration (HCC) in mountain goats, at Caw Ridge, Alberta (2001-2016). The dots represent annual population averages with standard error of FGM and HCC after accounting for age, sex, and individual identity. Log-transformed HCC averages with standard errors are presented to allow same-scale comparison with FGM.

Figure 2.4 Direct and indirect relationships between annual environmental variables (predation risk, resource availability measured as faecal crude protein peak, and population size), physiological stress estimated at the population level (**A**: faecal glucocorticoid metabolites concentration, n=761 over 16 years, **B**: hair cortisol concentration, n=511 over 20 years), and annual reproduction of mountain goat females at Caw Ridge, Alberta (1994-2016). Standardized path coefficients [95% CIs] are presented above each path. Thick solid lines represent strong evidence of an effect (95% CIs do not include 0), thin solid lines represent weak evidence (95% CIs marginally include 0), and dotted lines indicate inconclusive relationships (95% CIs widely overlapping 0 in both directions). The models were consistent with the structure of the data (**A**: Fisher's $C_2 = 0.151$, p-value=0.93, **B**: Fisher's $C_2 = 0.308$, p-value=0.86).

Figure 2.5 Influence of relative predator occurrence on faecal glucocorticoid metabolites concentration (FGM) (**A**), and influence of FGM on the proportion of reproductive females (**B**), in mountain goats, at Caw Ridge, Alberta (1994-2016). The full lines represent the mean model predictions and are presented with their 95% CI (**A**: dotted lines, **B**: polygon). The dots and error bars represent the mean and standard error in annual FGM predicted after accounting for age, sex, and individual identity. The shaded violin (**A**) shows the distribution of the raw data. Numbers are sample sizes, and the hollow dot represents the year with a single sample which was excluded from the analyses, but plotted to show consistency.

Figure 2.S1 Mass adjusted to July 15th in relation to age in mountain goats, at Caw Ridge, Alberta (1994-2016). The dots represent the average of all adjusted masses available for each individual-year. The lines are the predictions of the best polynomial regression of mass on age for males, non-reproductive females and reproductive females. Residuals were used as a measure of age-specific mass.

Figure 2.S2 Variation in faecal glucocorticoid metabolites concentration (FGM) during summer in mountain goats, Caw Ridge (Alberta; 2000-2016). Dot size is proportional to sample size, ranging from 1 to 30. The fitted quadratic regression is presented with its 95% CI, including age class and sex as fixed covariates, and goat identity and year as random intercepts. Reproduced with permission from Dulude-de Broin et al., 2019 in *General and Comparative Endocrinology*.

Figure 2.S3 Direct and indirect relationships between annual environmental variables (predation risk, resource availability measured as faecal protein peak, and population size), physiological stress (faecal glucocorticoid metabolites, n=697 over 15 years) estimated at the population level when accounting for variation in individual's age-specific mass, and annual reproduction, in mountain goats at Caw Ridge, Alberta (2001-2016). Standardized path coefficients [95% CIs] are presented above each path. Thick solid lines represent strong evidence of an effect (95% CIs do not include 0), thin solid lines represent weak evidence of an effect (95% CIs marginally include 0), dotted lines indicate inconclusive relationships (95% CIs widely overlapping 0 in both directions). The model was consistent with the structure of the data (Fisher's $C_2 = 0.308$, p-value=0.86).

Figure 2.S4 Direct and indirect relationships between annual environmental variables (predation risk, resource availability measured as faecal crude protein peak, and population size), physiological stress estimated at the population level with a data set limited to samples from females (**A**: faecal glucocorticoid metabolites concentration, **B**: hair cortisol concentration), and annual reproduction of mountain goat females at Caw Ridge, Alberta (1994-2016). Standardized path coefficients [95% CIs] are presented above each path. Thick solid lines represent strong evidence of an effect (95% CIs do not include 0), thin solid lines represent weak evidence (95% CIs marginally include 0), and dotted lines indicate inconclusive relationships (95% CIs widely overlapping 0 in both directions). The models were consistent with the structure of the data (**A**: Fisher's $C_2 = 0.157$, p-value=0.93, **B**: Fisher's $C_2 = 0.293$, p-value=0.86).

Figure 2.S5 Influence of relative predator occurrence on faecal glucocorticoid metabolites concentration (FGM) estimated with a data set limited to samples from females (**A**), and influence of FGM on the proportion of reproductive females (**B**), in mountain goats, at Caw Ridge, Alberta (2001-2016). The full lines represent the mean model predictions and are presented with their 95% CI (**A**: dotted lines, **B**: polygon). The dots and error bars represent the mean and standard error in annual FGM predicted with female samples after accounting for age, and individual identity. The shaded violin (**A**) shows the distribution of the raw data. Numbers are sample sizes, and the hollow dot represents the year with a single sample.

Figure 2.S6 Direct and indirect relationships between annual environmental variables (predation risk, resource availability measured as faecal protein peak, and population size),

physiological stress measured at the individual level (**A**: faecal glucocorticoid metabolites concentration, n=83 over 15 years, **B**: hair cortisol concentration, n=36 over 11 years), and individual reproductive success, in mountain goats at Caw Ridge, Alberta (1994-2016). Standardized path coefficients [95% CIs] are presented above each path. Thick solid lines represent strong evidence of an effect (95% CIs do not include 0), thin solid lines represent weak evidence of an effect (95% CIs marginally include 0), dotted lines indicate inconclusive relationships (95% CIs widely overlapping 0 in both directions). The models were consistent with the structure of the data (**A**: Fisher's $C_{10} = 10.55$, p-value=0.39, **B**: Fisher's $C_8 = 11.44$, p-value=0.18).

Liste des tableaux

Table 1.1 Model selection for the influence of age class, sex and reproductive status on faecal glucocorticoid metabolite concentration and hair cortisol concentration in mountain goats, at Caw Ridge, Alberta (2000-2016). First set of candidate models (a) is based on the full data set (nfaeces=761; nhair=511), second set (b) is based on a subset of adult females for which reproductive status was known (nfaeces=271; nhair=115). Individual identity and year were included in all models as random factors. K is the number of parameters. AICc wt is the relative weight of the model compared to other candidate models.

Table 1.S1 Model selection for the influence of date of collection on faecal glucocorticoid metabolite concentration in mountain goats, at Caw Ridge, Alberta (2000-2016). All models included age class and sex as fixed covariates and goat identity and year as random factors. K is the number of parameters.

Table 1.S2 Multiple comparisons of grouping patterns for the effect of age class on hair cortisol concentration in mountain goats, at Caw Ridge, Alberta (2000-2016), using model selection with Akaike information criterion corrected for small sample size (AICc). K is the number of parameters.

Table 2.S1 Means and standard deviations (SD) used to standardized the variables included in a) the population path analyses, b) the individual path analyses.

Liste des abréviations

- ACTH : Adrénocorticotrophine / Adrenocorticotropic hormone
AIC: Critère d'information d'Akaike / Akaike information criterion
CRH : Corticolibérine / Corticotropin-releasing hormone
EIA : Dosage immuno-enzymatique / Enzyme Immunoassay
FGM : Métabolites fécaux de glucocorticoïde / Faecal glucocorticoid metabolite
GC : Glucocorticoïde/Glucocorticoid
HCC : Concentration en cortisol dans les poils / Hair cortisol concentration
HPA : Axe hypothalamo-hypophysaire / Hypothalamic-pituitary-axis
HPLC : Chromatographie en phase liquide inverse à haute performance / Reverse phase high performance liquid chromatography
IU : Unité internationale / International Unit
SAM : Axe sympatho-surrénalien / Sympathoadrenal medullary axis

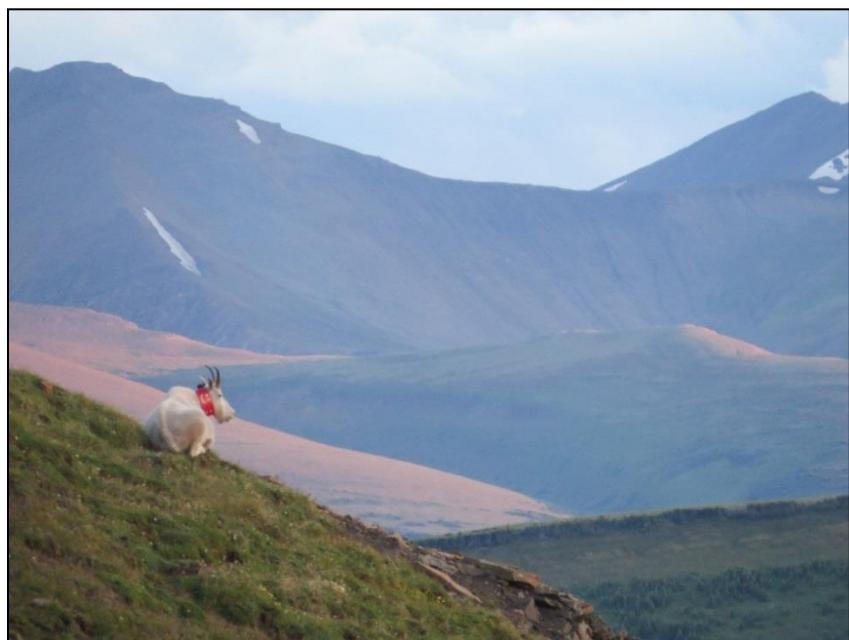
Le 12 août 2016, au West-End de Caw Ridge.

Dans le vestibule des montagnes millénaires qui forment les Rocheuses se trouve une succession de quatre sommets comme le torse d'autant de géants qui, la tête dans les nuages, auraient de larges épaules s'étendant vers le sud et vers le nord. Protégé du rythme fou des sociétés humaines par l'altitude et la rigueur de l'hiver, le noble relief n'en est pas moins gorgé de vie. Mille chiens de prairie, marmottes et tamias y ont dessiné un vaste réseau souterrain qui s'ouvre sur des pièces secrètes où eux seuls peuvent tenir conseil. Aigles, buses et faucons font affront à l'orgueil des géants en se laissant couler dans les sentiers de vent qui, complices, les portent bien au-delà du sol et de ses crêtes. Une famille de renard aux poils vifs comme ces feux qui ravagent les forêts de basses altitudes a élu domicile dans une butte de pierre certes modeste, mais offrant une vue imprenable sur le vaste pays. Une fois aménagé, l'endroit sera accueillant et chaleureux, les trois renardeaux y jouent d'ailleurs déjà à la cachette tout l'après-midi. Le tendre velours vert déposé comme une nappe sur le relief n'a rien de prévisible et monotone comme ce que la courte saison estivale pourrait laisser croire, mais se pare plutôt des couleurs les plus guillerettes dès la venue du printemps. Ce sont par centaines que les frêles pousses annuelles habillent la montagne d'un manteau floral auquel jour après jour s'ajoutent de nouvelles broderies. Vers la mi-août, au temps où le mauve et le fuchsia sont à la mode, quelques ours grimpent sur les plateaux et fouillent le sol de leurs larges pattes afin d'en extraire les gouteuses racines; du haut d'une paroi escarpée, la silhouette vigilante d'un mouflon aux nobles cornes spiralées guette les mouvements de ces faux végétariens.

Assis à flanc de montagne, caché par un éboulis rocheux et réchauffé par le thé brûlant et trop sucré qui emplit mon thermos, j'observe celles qui ne se laissent pas voir. Glissant sur le paysage comme autant d'ombres blanches, elles semblent être l'âme et les gardiennes de ces lieux. Coiffées d'élégantes cornes noires en diadème et vêtues d'une riche chasuble ivoire, elles avancent d'un pas assuré sur le fil d'une paroi rocheuse. Ayant plusieurs fois bravé les hostilités de l'hiver, le caractère de ces dames diaphanes contraste avec l'uniformité de leur robe. Il y a 308, matriarche redoutable et respectée, toujours accompagnée de sa fille 448, craintive et sans orgueil, tentant une fois de plus, malgré son âge plus qu'avancé, d'obtenir le nectar lacté de celle qui lui a donné vie. Il y a 458, petit mâle libidineux qui, à deux ans, essaie déjà ses charmes sans grands succès auprès de celles qui auraient pu être 4 fois sa mère. Il y a 333 aux cornes dressées comme des antennes de télévision; 418 acrobate frivole et mère aimante d'un fils dodu; 431 dont l'appétit n'a d'égal que son manque d'intérêt pour les enfants; 454 qui, orpheline à 1 an, est aujourd'hui rusée et pleine de ressources; 413 dont le patron de mue semble tout droit sorti du catalogue d'un barbier excentrique du Mile-

End; et 440, petite demoiselle aux traits fins remportant assurément, s'il existe, le concours annuel de beauté caprin. Enfin, il y a 341, habitée d'une douce folie, ouverte d'esprit et téméraire qui déguste les larges feuilles vertes des vallées en prenant le thé avec les grizzlys. Tout aussi uniques qu'elles soient, ses âmes se connaissent et se côtoient depuis l'enfance, et c'est ainsi qu'unies dans leurs différences, elles mangent, se déplacent et sommeillent à l'unisson.

Les voilà justement qui se lèvent. C'est le moment où j'entre en jeu. À mesure qu'elles quittent Morphée pour se joindre à Bacchus, je fige leur position en 2 ou 3 détentes de caméra et les observe s'éloigner progressivement, rapetissant d'une bouchée à l'autre. Une fois hors de vue, je démonte mon télescope et, mon sac sur le dos, je délaisse ma colline, descends dans la vallée et remonte sur la leur. Je reconstitue alors chacune des fresques dont j'ai la photo et je récolte les témoins physiologiques qui me permettront de mieux comprendre ces fantômes alpins. L'après-midi est déjà bien avancée et le soleil, gêné d'avoir été surpris à bâiller, rougit légèrement avant d'amorcer sa descente derrière les montagnes. Concentré sur mes échantillons et les données qu'ils représentent, je marche le nez au sol, oubliant un peu les chèvres. Au moment où je relève la tête, j'aperçois 354 paisiblement couchée dans l'herbe et le poil teinté par les derniers rayons du soleil qui s'agencent doucement à la couleur de son collier. Inconsciente de ma présence, la splendeur du paysage et la tranquillité des lieux semblent l'habiter complètement. Je délaisse un peu mon sac et me couche dans l'herbe pour l'admirer en me disant qu'en dehors des données et de l'intérêt scientifique, notre rôle est peut-être un peu aussi d'être ces témoins privilégiés qui assistent aux spectacles intimes des mondes reculés et en rapportent les paysages.



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Avant-propos

Ce mémoire débute par une introduction générale présentant le contexte théorique dans lequel s'insère mon projet, suivie de deux chapitres rédigés en anglais sous forme d'articles scientifiques et d'une conclusion rappelant les résultats principaux du mémoire et discutant leurs implications.

Chapitre 1. Faecal metabolites and Hair cortisol as biological markers of HPA-axis activity in the Rocky Mountain Goat.

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Auteur principal : Frédéric Dulude-de Broin (FDB).

Coauteurs : Steeve D. Côté (SC), Douglas P. Whiteside (DW), Gabriela F. Mastromonaco (GM).

Contribution des auteurs : FDB, GM et SC ont défini le projet. FDB et DW ont effectué les manipulations expérimentales. FDB, DW et SC ont collecté les échantillons. FDB et GM ont réalisé les analyses en laboratoire. FDB a effectué les analyses statistiques. FDB a rédigé l'article. GM et SC ont révisé plusieurs versions du manuscrit.

Chapitre 2. Predation risk and mountain goat reproduction: Evidence for stress-induced breeding suppression in a wild ungulate.

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Auteur principal : Frédéric Dulude-de Broin (FDB) Coauteurs : Sandra Hamel (SH), Gabriela F. Mastromonaco (GM) et Steeve D. Côté (SC)

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Introduction

Les effets directs et indirects de la prédation

La prédation est un élément central de la dynamique de population des espèces proies. À première vue, le mode d'action semble simple. Plus les prédateurs capturent et consomment de proies, plus la taille de la population de proies diminue. En raison de ses effets marqués sur la survie, cette action directe de la prédation a été largement étudiée (Heithaus et al., 2008; Mesa et al., 1994; Schmitz et al., 2000; Sih et al., 1985) et fut longtemps considérée comme le principal aspect de la dynamique prédateur-proie (Peckarsky et al., 2008; Sih et al., 1985; Volterra, 1928). Cette vision a été remise en question par les résultats étonnantes d'expériences conçues de manière à éliminer l'action directe de la prédation tout en conservant la perception du risque pour les proies. Schmitz et al. (1997) ont particulièrement bien illustré ces résultats en comparant deux groupes de sauterelles (*Melanoplus femur-rubrum*) exposés à différentes conditions de prédation. Le premier groupe était exposé à des araignées (*Pisurina mira*) non manipulées, alors que le second faisait face à des araignées dont les chélicères avaient été collées afin de les empêcher de blesser ou de capturer les proies. Le groupe de sauterelles exposé aux prédateurs invalides avait un taux de mortalité équivalent à celui des sauterelles exposées au prédateur valide parce que le risque de prédation limitait leur capacité à s'alimenter (Schmitz et al., 1997). La simple présence d'un prédateur au sein d'un écosystème, en dehors de la mortalité directe qu'elle induit, peut donc avoir des conséquences majeures pour une population de proies. Ces effets indirects de la prédation résultent des stratégies qu'adoptent les proies pour ne pas être tuées (Clinchy et al., 2013; Heithaus et al., 2008; Lima, 1998; Lima et Dill, 1990).

La plupart des proies adoptent des stratégies de défense face à la prédation (Preisser et al., 2005; Werner et Peacor, 2003). Ces stratégies se traduisent notamment par des changements comportementaux (Lima et Dill, 1990), morphologiques (Werner et Peacor, 2003) et physiologiques (Slos et Stoks, 2008). Par exemple, lorsque le risque de prédation est élevé, les dauphins (*Tursiops aduncus*) sélectionnent des habitats en profondeur où ils sont à l'abri de leurs prédateurs (Heithaus et Dill, 2006), les daphnies (*Daphnia sp.*) développent des structures morphologiques défensives qui diminuent le risque qu'elles soient consommées (Grant et Bayly, 1981; Tollrian, 1995) et la plupart des proies entrent en état d'alerte

physiologique, modifiant leur métabolisme afin de permettre une mobilisation optimale des ressources vers les fonctions essentielles pour survivre à la menace (Hawlena et Schmitz, 2010; Wingfield et al., 1998). À court terme, ces adaptations sont avantageuses puisqu'elles augmentent la probabilité qu'un individu échappe à ses prédateurs, mais elles constituent toujours un compromis au niveau de l'acquisition et de l'allocation des ressources dont dispose l'animal (Werner et Peacor, 2003). En reprenant les exemples précédents, les habitats en profondeur sont moins risqués, mais également moins riches en nourriture que ceux en surface (Heithaus et Dill, 2006), l'augmentation des dépenses énergétiques engendrées par le développement de structures défensives réduit le succès reproducteur et la valeur adaptative globale des daphnies (Barry, 1994) et l'allocation préférentielle des ressources se fait au détriment d'autres fonctions physiologiques, inhibant par exemple la croissance et la reproduction (Hawlena et Schmitz, 2010). Contrairement à la mortalité directe qui affecte généralement un nombre restreint d'individus au sein d'une population (ceux qui sont tués), les coûts associés aux stratégies anti-prédation affectent l'ensemble des individus qui réagissent à la menace. Au niveau de la population, les conséquences de ces effets indirects de la prédation sur les proies peuvent être aussi marquées que celles associées à la consommation d'individus (Preisser et al., 2005).

Le mécanisme incertain des effets indirects de la prédation

Bien que les effets indirects de la prédation soient maintenant largement reconnus comme un aspect essentiel des interactions prédateurs-proies (Clinchy et al., 2013; Lima, 1998; Luttbeg et Kerby, 2005; Peacor et Werner, 2001; Peckarsky et al., 2008), le mécanisme général qui lie la perception du risque par la proie à ses conséquences démographiques demeure incertain (Creel, 2018). Deux hypothèses principales ont été proposées. La première soutient que les comportements anti-prédation favorisent la sécurité de la proie au détriment de l'acquisition de nourriture, ce qui limite les ressources énergétiques disponibles pour la reproduction et la survie (ci-après *Hypothèse prédation-sélection de ressources*, de l'anglais *Predation-sensitive foraging hypothesis*; Hik, 1995; Sinclair et Arcese, 1995). Cette hypothèse est abondamment supportée par la littérature tant au niveau expérimental, qu'en milieu naturel (Brown et Kotler, 2004; Christianson et Creel, 2010; Creel et al., 2014; Fortin et al., 2005; Lima, 1998; Sih, 1980; Zanette et al., 2003). Toutefois, elle ne parvient pas à expliquer la

totalité des coûts démographiques associés au risque de prédatation (p.ex. Boonstra et al., 1998; Krebs et al., 1995), et donc, une seconde hypothèse a été proposée selon laquelle le risque de prédatation agit sur la survie et la reproduction en induisant une élévation chronique du stress chez la proie (ci-après *Hypothèse prédatation-stress*, de l'anglais *Predation-stress hypothesis*; Boonstra et al., 1998; Clinchy et al., 2013).

L’homéostasie, l’allostasie et le stress

La survie d’un organisme repose sur sa capacité à maintenir son homéostasie, c’est-à-dire la stabilité de ses paramètres physiologiques fondamentaux comme la température, le pH et la glycémie au sein d’un environnement variable (McEwen et Wingfield, 2003). Cet état de stabilité qu’est l’homéostasie est soutenu par l’allostasie, un processus qui entraîne la modulation d’autres paramètres physiologiques tels que la pression artérielle ou les concentrations d’hormones et de neurotransmetteurs (McEwen et Wingfield, 2003). Ainsi, en réponse aux fluctuations de l’environnement ou à un changement de stade d’histoire de vie, la modulation de certains paramètres physiologiques (allostasie) permet de répondre aux nouvelles contraintes subies par l’organisme tout en maintenant la stabilité des paramètres physiologiques fondamentaux essentiels à sa survie (homéostasie).

Le stress est un mécanisme clef de l’allostasie qui comprend l’ensemble des réactions neuroendocrines favorisant la survie immédiate d’un organisme et le retour à l’homéostasie (Sheriff et al., 2011b). La réponse au stress est déclenchée lorsqu’un stimulus physique ou psychologique, un facteur de stress, perturbe l’homéostasie. Chez les vertébrés, la réponse au stress implique deux axes de contrôle hormonal. Le premier axe, l’axe sympatho-surrenalien (axe SAM), réagit en quelques dixièmes de secondes et provoque la libération de catécholamines sous forme d’adrénaline et de noradrénaline (Reeder et Kramer 2005). Ces hormones placent immédiatement le corps en état d’alerte et entraînent notamment une élévation rapide du rythme cardio-respiratoire et de la circulation sanguine afin d’offrir une perfusion maximale au cerveau, au cœur et aux muscles squelettiques (Reeder et Kramer 2005). Le second axe de réaction, l’axe hypothalamo-hypophyso-surrénalien (axe HPA; Sapolsky et al., 2000; Wingfield et Romero, 2001), réagit en quelques minutes et provoque la libération en cascade de corticolibérine (CRH), puis d’adrénocorticotrophine (ACTH).

L'ACTH stimule alors la production de glucocorticoïdes (GCs) majoritairement sous forme de cortisol ou de corticostérone selon l'espèce.

Les GCs sont des hormones métaboliques impliquées dans la régulation de la balance énergétique au quotidien (Landys et al., 2006), qui ont également un rôle essentiel dans la gestion de l'énergie lorsqu'un événement met en péril la survie de l'organisme (Sapolsky et al., 2000). Au niveau basal, les GCs régissent le rythme circadien et mobilisent les ressources énergétiques d'un animal en synchronie avec ses besoins saisonniers et ses stades d'histoire de vie (Dallman et al., 1993; Landys et al., 2006; Romero, 2002). À haute concentration, les GCs mobilisent l'énergie vers les fonctions d'urgence de l'organisme en stimulant la production de glucose par néoglucogenèse, en modulant la réponse immunitaire et en détournant les ressources énergétiques des fonctions à long terme comme la croissance et la reproduction (Sapolsky et al., 2000; Wingfield et al., 1998).

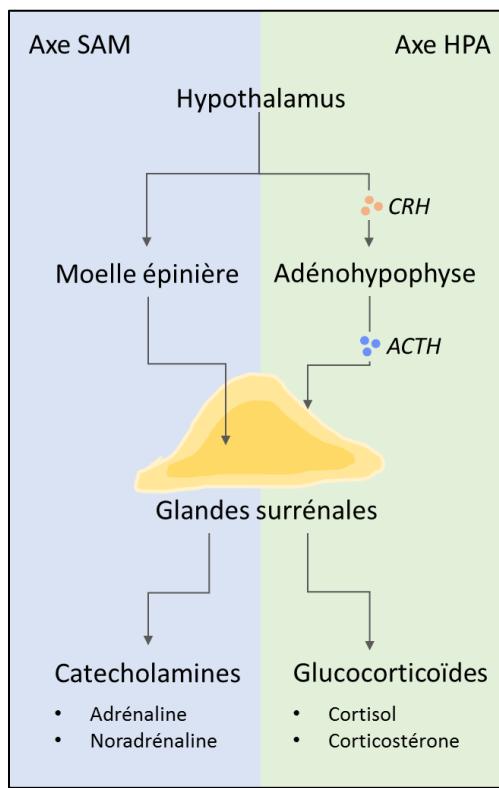


Figure 0.1 Réactions neuroendocrines des deux axes de réponse au stress suite à la détection d'un facteur de stress. L'axe sympatho-surrenalien (SAM) stimule la production de catécholamines par les glandes surrenales, alors que l'axe hypothalamo-hypophysaire-surrenalien (HPA) entraîne la production de glucocorticoïdes via la libération en cascade de corticotéline (CRH) et d'adrénocorticotrophine (ACTH).

L'objectif général de la réponse au stress est d'assurer un apport énergétique adéquat aux organes impliqués dans la réaction de survie et d'éviter que d'autres fonctions compétitionnent pour cette énergie (Wingfield et al., 1998). À court terme, ce mécanisme permet à un organisme d'augmenter la probabilité qu'il survive à une menace. Cependant, l'activation chronique ou soutenue de la réponse au stress peut fortement nuire à la condition physique (Sheriff et al., 2011b, 2009), la survie (MacLeod et al., 2018; Pride, 2005) ou la reproduction (Boonstra et al., 1998; Sheriff et al., 2009) et avoir des conséquences démographiques majeures pour une population (Boonstra et al., 1998). L'élévation des concentrations de glucocorticoïdes interfère notamment de manière directe avec les hormones qui stimulent la croissance, le maintien et la reproduction (Tsigos et Chrousos 2002, Breen et Mellon 2014) et engendre des dépenses énergétiques supplémentaires réduisant l'énergie disponible pour les fonctions à long terme (Hawlena et Schmitz 2010).

L'hypothèse prédation-stress

La réponse au stress est un élément central de la réponse d'une proie faisant face à la menace immédiate que représente l'attaque d'un prédateur (Hawlena et Schmitz 2010). D'après l'*hypothèse prédation-stress* l'action indirecte de la prédation sur la reproduction ou la survie provient principalement des effets nocifs liés à l'activation chronique ou soutenue de la réponse aux stress. L'*hypothèse prédation-stress* est plus récente et a par conséquent été moins étudiée que l'*hypothèse prédation-sélection de ressources*, mais elle est supportée par un nombre croissant d'études empiriques (Clinchy et al., 2013; Hammerschlag et al., 2017; Hawlena et Schmitz, 2010; Yin et al., 2017) et semble avoir un rôle central dans les imposantes fluctuations démographiques associées aux cycles lièvres (*Lepus americanus*)-lynx (*Lynx canadensis*) en forêt boréale (Boonstra et al., 1998; MacLeod et al., 2018; Sheriff et al., 2011b, 2010, 2009). Toutefois, dans certains systèmes le risque de prédation ne semble pas induire de stress physiologique chronique chez la proie, et ce même lorsqu'il entraîne un déclin de la reproduction (Fontaine et al., 2011; Ylönen et al., 2006). Par exemple, la fertilité des femelles wapiti (*Cervus canadensis*) du parc National de Yellowstone (É.-U.) a décliné suite à la réintroduction du loup gris (*Canis lupus*) dans l'écosystème (Creel et al., 2007), mais ces effets indirects de la prédation sont principalement expliqués par l'*hypothèse prédation-sélection de ressources* (Christianson et Creel, 2010; Fortin et al., 2005; Ripple et

Beschta, 2004) sans appui pour l'*hypothèse prédation-stress* (Creel et al., 2009). La comparaison de systèmes où l'*hypothèse prédation-stress* est supportée et où elle ne l'est pas est indispensable à la compréhension des mécanismes à l'origine des effets indirects de la prédation.

La chèvre de montagne, un ongulé alpin comme modèle d'étude

Les ongulés alpins sont des espèces intéressantes pour tester l'*hypothèse prédation-stress* car ils pourraient être limités dans leur capacité à prédire et contrôler leur exposition au risque. Ils sont généralement distribués en populations de petite taille, ne pouvant soutenir à elles seules une population de prédateurs (Festa-Bianchet et al., 2006). Le nombre de prédateurs varie donc en fonction de l'abondance de proies alternatives, ce qui peut engendrer des fluctuations rapides et imprévisibles du risque de prédation (Festa-Bianchet et al., 2006; Rominger et al., 2004; Wittmer et al., 2005). De plus, les ongulés alpins sont généralement confinés sur des îlots montagneux entourés d'habitats non favorables (Festa-Bianchet, 1991; Festa-Bianchet et al., 1994). Le manque d'habitats alternatifs pourrait ainsi les contraindre spatialement, les empêchant de contrôler leur exposition au risque par l'utilisation d'habitats plus sécuritaires (Schmidt et Kuijper, 2015). Des expériences en laboratoire ont démontré que la réaction d'un animal face à un facteur de stress devient plus intense lorsque l'événement perturbateur est imprévisible ou incontrôlable (Dess et al., 1983; Hanson et al., 1976; Weiss, 1970). La capacité limitée des ongulés alpins à prédire et contrôler leur exposition au risque de prédation pourrait favoriser le stress comme mécanisme des effets indirects de la prédation (Creel, 2018).

La chèvre de montagne (*Oreamnos americanus*) est un ongulé alpin retrouvé exclusivement dans les montagnes de l'ouest de l'Amérique du Nord. Cette espèce est longévive et vit fréquemment plus de 10 ans en milieu naturel (Festa-Bianchet et Côté, 2008). L'âge de primiparité est tardif et les femelles ne produisent généralement qu'un seul chevreau par année en condition naturelle (Festa-Bianchet et Côté, 2008). Le succès reproducteur à vie des femelles est hautement corrélé à la longévité (0.91; Panagakis et al., 2017). Conséquemment, elles adoptent une stratégie de reproduction hautement conservatrice (Hamel et al., 2010) et ne compromettent pas leur survie pour un événement de reproduction particulier (Festa-

Bianchet et Côté, 2008; Hamel et al., 2010), prenant régulièrement des pauses reproductives en faveur de leur condition corporelle (Festa-Bianchet et Côté, 2008). La prédation est le principal facteur de mortalité chez les populations indigènes de chèvres de montagne et est susceptible d'être fortement contrastée d'une année à l'autre (Festa-Bianchet et Côté, 2008). En milieu naturel, les principaux prédateurs de la chèvre de montagne sont le cougar (*Puma concolor*), le loup gris et l'ours grizzly (*Ursus arctos*). L'ours noir (*Ursus americanus*), le coyote (*Canis latrans*), le carcajou (*Gulo gulo*) et l'aigle royal (*Aquila chrysaetos*) représentent aussi des menaces potentielles.

La population de Caw Ridge

La population de chèvres de montagne de Caw Ridge se trouve dans les montagnes Rocheuses du centre-ouest de l'Alberta (Canada). Elle occupe une aire d'environ 28 km² composée de toundras alpine et subalpine à une altitude variant entre 1700 et 2180 mètres. La population de Caw Ridge est isolée des autres populations de chèvres de montagne par de vastes étendues de forêt boréale sans habitats profitables pour les chèvres. L'émigration et l'immigration sont extrêmement rares (Festa-Bianchet et Côté, 2008). La chasse est interdite depuis 1969 (Festa-Bianchet et Côté, 2008). La quasi-totalité de la population est marquée et un suivi intensif effectué chaque ét permet de déterminer le succès reproducteur et la survie de chaque individu, en plus de plusieurs caractéristiques individuelles telles que l'âge, le sexe, le rang social et la masse. Le risque de prédation est estimé d'après l'occurrence relative de prédateurs aperçus sur l'aire d'étude durant l'été. L'ensemble des prédateurs de la chèvre de montagne mentionnés précédemment peuvent être observés à Caw Ridge.

La population de Caw Ridge a subi de grandes variations démographiques au cours des dernières années. La taille de la population a augmenté de 81 individus en 1990 à 152 en 2003, est restée relativement stable jusqu'en 2008 et a ensuite décliné de 80% pour atteindre 34 individus en 2017. La proportion de femelles reproductives variait autour de 50% entre 1989 et 2002, puis a progressivement diminué fluctuant autour de 20% entre 2010 et 2017. La présence relative de prédateurs sur l'aire d'étude a augmenté récemment avec les sept valeurs annuelles les plus élevées obtenues au cours des onze dernières années.

Note sur la mesure du stress en milieu naturel

L'évaluation du stress physiologique d'un animal a traditionnellement été effectuée par la mesure des concentrations en glucocorticoïdes dans le sang ou le plasma (Sheriff et al., 2011a). Cependant, la fluctuation rapide des taux d'hormones sanguins (Windle et al., 1998) et l'élévation des concentrations en glucocorticoïdes lors des captures, contentions et prises de sang (Müller et al., 2006; Widmaier et Kunz, 1993) ont entraîné le développement de méthodes utilisant des matrices biologiques alternatives au sang comme les fèces et les poils. La concentration en métabolites de glucocorticoïdes dans les fèces représente une mesure intégrée du stress physiologique durant le temps de transit intestinal (généralement de 1 à 48 heures selon l'espèce; Palme et al., 2005, 1996) et est largement utilisée en écologie animale (Palme, 2019). Le type de métabolites formés, ainsi que le délai et la voie d'excrétion (urine ou fèces) peuvent toutefois grandement varier selon les espèces, l'âge ou le sexe (Palme et al., 2005; Touma et al., 2003; Touma et Palme, 2005). Les poils ont quant à eux le potentiel d'intégrer l'état physiologique d'un animal durant plusieurs mois (Heimbürge et al., 2019; Koren et al., 2019). Bien que la voie d'incorporation des glucocorticoïdes dans cette matrice demeure incertaine, l'hypothèse la plus plausible est que les hormones sont incorporées à partir des capillaires sanguins qui entourent le follicule lors de la période de croissance active du poil (Harkey, 1993; Pragst et Balikova, 2006). Toutefois, certaines études suggèrent qu'une quantité non négligeable de glucocorticoïdes pourraient se retrouver dans les poils indépendamment des concentrations d'hormones dans le sang (Keckeis et al., 2012), notamment *via* la production locale de glucocorticoïdes au niveau des follicules pileux (Ito et al., 2005). En somme, alors que l'utilité des fèces comme marqueurs physiologiques est bien établie dans la littérature (Palme, 2019), les poils sont à la frontière des connaissances techniques en écophysiologie (Koren et al., 2019; Sheriff et al., 2011a).

La mesure des glucocorticoïdes au sein de matrices biologiques alternatives au sang est une avenue prometteuse pour étudier les populations animales en milieu naturel, mais la validité de ces méthodes repose sur le principe que la concentration en glucocorticoïdes dans la matrice d'intérêt reflète l'état physiologique de l'animal (Sheriff et al., 2011a; Touma et Palme, 2005). Cette prémissse doit être vérifiée pour chaque espèce et chaque matrice biologique considérées (Buchanan et Goldsmith, 2004; Sheriff et al., 2011a; Touma et Palme,

2005). L'une des méthodes de validation privilégiée est d'effectuer un test de stimulation à l'adrénocorticotrophine (ACTH; Buchanan et Goldsmith, 2004; Sheriff et al., 2011a; Touma et Palme, 2005). Puisque l'ACTH stimule la production de glucocorticoïdes par l'axe hypothalamo-hypophysio-surrénalien, une méthode de mesure est considérée valide si elle permet de détecter une augmentation en glucocorticoïdes suivant l'administration d'ACTH exogène. L'utilisation des fèces comme marqueur du stress physiologique n'a encore jamais été validée chez la chèvre de montagne et l'utilisation des poils n'a jamais été validée chez un ongulé non domestique (mais voir Ashley et al. (2011) pour une tentative chez le caribou (*Rangifer tarandus*)).

Objectifs

À travers ce mémoire, mon projet vise à contribuer à la compréhension des effets indirects de la prédation en milieu naturel en utilisant la chèvre de montagne comme modèle biologique. Bénéficiant de 23 ans de données longitudinales sur une population d'individus marqués et suivis durant toute leur vie en milieu naturel, nous avons testé l'*hypothèse prédation-stress* en évaluant si le risque de prédation influence la reproduction de la chèvre de montagne par l'induction d'un état de stress chronique. Dans un premier temps, nous avons validé l'utilisation des fèces et des poils comme marqueurs du stress physiologique chez cette espèce et évalué l'effet confondant de plusieurs variables individuelles et méthodologiques sur la mesure des glucocorticoïdes. Nous avons ensuite testé la prédiction selon laquelle un haut risque de prédation est associé à une élévation des concentrations en glucocorticoïdes, lesquelles sont associées à une faible proportion de femelles reproductives.

1. Faecal metabolites and Hair cortisol as biological markers of HPA-axis activity in the Rocky Mountain Goat

Frédéric Dulude-de Broin^{* a}, Steeve D. Côté^a, Douglas P. Whiteside^{c,d} and
Gabriela F. Mastromonaco^b

^a Département de biologie, and Centre d'études Nordiques, Université Laval, Québec,
Canada, G1V 0A6

^b Reproductive Physiology, Toronto Zoo, 361A Old Finch Avenue, Toronto, Ontario,
Canada, M1B 5K7

^c Department of Ecosystem and Public Health, Faculty of Veterinary Medicine, University
of Calgary, Alberta, Canada, T2N 4Z6

^d Calgary Zoo, Alberta, Canada, T2E 7V6

^{*} Corresponding author: frederic.ddb@gmail.com

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1.1 Résumé

La mesure des glucocorticoïdes dans les fèces et les poils est un moyen puissant et minimalement intrusif d'évaluer l'état physiologique des animaux en milieu naturel. Ces méthodes doivent cependant être validées pour chaque nouvelle espèce et chaque matrice biologique analysée. Nous avons effectué un test de stimulation à l'adrénocorticotrophine (ACTH) *via* l'injection hebdomadaire d'ACTH durant 5 semaines afin d'évaluer la validité des fèces et des poils comme marqueurs biologiques du stress physiologique chez la chèvre de montagne (*Oreamnos americanus*). Nous avons également évalué l'effet de facteurs endogènes (âge, sexe, statut reproducteur) et méthodologiques (date de collection et délai de congélation des fèces, type de poil) sur les mesures hormonales à partir d'échantillons collectés au cours d'une étude longitudinale sur une population sauvage de chèvres de montagne. Notre méthode de mesure était adéquate pour les fèces et les poils, et suffisamment sensible pour détecter une nette élévation des glucocorticoïdes au sein des deux matrices biologiques suite à l'injection d'ACTH. L'âge et le sexe n'avaient pas d'effet détectable sur les concentrations mesurées dans les fèces, mais la concentration en cortisol dans les poils était plus élevée chez les chèvres de 1 an et moins que chez les chèvres plus âgées, et plus faible chez les mâles adultes que les femelles adultes. L'effet potentiel du statut reproducteur sur les concentrations de glucocorticoïdes était imperceptible dans les fèces et dans les poils. La concentration fécale en métabolites de glucocorticoïdes augmentait avec la date de collection de l'échantillon de la fin mai à la mi-juin et diminuait ensuite jusqu'à la mi-septembre. Les poils de garde avaient une concentration en cortisol environ deux fois plus élevée que les poils de bourre. La concentration en métabolites fécaux diminuait lorsque le délai de congélation était prolongé, mais la dégradation semblait limitée lorsque l'échantillon était exposé au vent et au soleil ou à de faibles températures. Nous concluons que les fèces et les poils peuvent être utilisés pour évaluer l'activité de l'axe hypothalamo-hypophysosurrénalien chez la chèvre de montagne à condition que les facteurs confondants soient considérés lors de l'interprétation des résultats.

1.2 Abstract

Monitoring glucocorticoids in faeces and hair is increasingly used in ecological studies and provides a powerful and minimally intrusive mean to identify physiological challenges faced by wild animals. Using a cortisol and a corticosterone immunoassays, we conducted an adrenocorticotrophic (ACTH) challenge with five weekly repeated injections to validate the use of faecal glucocorticoid metabolites and hair cortisol concentration as biological markers of the HPA-axis activity in captive mountain goats (*Oreamnos americanus*). We also investigated the effect of endogenous (age, sex, reproductive status) and methodological (faecal sample collection dates, freezing delay and hair types) variables on glucocorticoid values using faecal and hair samples collected from marked wild mountain goats during a long-term study. The cortisol enzyme immunoassay was reliable for mountain goat faeces and hair, and was sensitive enough to detect a clear rise in glucocorticoid concentrations following ACTH injections for both substrates. Age and sex had no detectable effect on faecal glucocorticoid metabolites, but hair cortisol concentration was higher in kids and yearlings than in older goats, and lower in adult males compared to adult females. Reproductive status had no detectable effect on both faecal and hair measurements. Faecal metabolites concentrations increased with sample collection date in late spring until mid-summer and decreased afterward until early fall. Guard hair had nearly twice as much cortisol per gram as undercoat hair. Prolonged delay to freezing reduced the concentration of faecal glucocorticoid metabolites, but degradation seemed limited when samples were exposed to wind and sun or when ambient temperature was low. We conclude that faeces and hair can be used as valid biomarkers of the HPA-axis activity in mountain goat provided that confounding variables are taken into account when interpreting measurements.

1.3 Introduction

One of the primary physiological processes helping vertebrates cope with challenges, such as seasonality and various forms of physiological disturbances, is the stimulation of the hypothalamic-pituitary-axis (HPA) leading to the release of glucocorticoid hormones (Landys et al., 2006; Reeder and Kramer, 2005b). Glucocorticoids (GCs; corticosterone/cortisol) are metabolic hormones involved in the maintenance of energetic balance that also act as key components of the acute stress response (Landys et al., 2006; Sapolsky et al., 2000). At baseline, GCs are drivers of the circadian rhythm and vary within the year to help animals meet seasonal needs (Dallman et al., 1993; Landys et al., 2006; Romero, 2002). Peak concentrations of GCs are reached as part of acute stress responses and redirect energy from long term functions towards functions required to rapidly cope with the stressor (Hawlena and Schmitz, 2010). While useful to avoid short term threats, prolonged periods of high glucocorticoid concentration may alter immune function, reproduction, and reduce survival (Wingfield and Sapolsky, 2003). Both baseline and stress-induced stimulation of the HPA axis are therefore informative of the life-history and environmental challenges faced by individuals (Landys et al., 2006; Reeder and Kramer, 2005b).

Glucocorticoid concentrations have traditionally been measured in blood serum or plasma, but pulsatile fluctuations (Windle et al., 1998) and capture/restraint increase of GC concentration in the blood (Müller et al., 2006; Widmaier and Kunz, 1993) have prompted the development of techniques using alternative biological matrices such as faeces and hair. Glucocorticoid metabolite levels in faeces provide a short-term integrated measure of the HPA axis activity during gut passage time (Palme, 2005; Palme et al., 1996). The types of metabolites formed, as well as the routes and time course of excretion can, however, differ greatly among species, sex or age-classes (Palme et al., 2005; Touma et al., 2003). Variation according to species, sex, and age must therefore be investigated before using faecal metabolites as an indicator of an animal's physiology. Hair, on the other hand, has the potential to record several months of glucocorticoid response. While not fully understood, the incorporation of hormones into hair is thought to occur during the period of active growth (i.e anagen) when the hair bulb is closely associated with the capillary system surrounding the follicles (Harkey, 1993; Pragst and Balikova, 2006), albeit follicular cells have been

shown to locally produce cortisol that does not necessarily reflect systemic concentrations (Ito et al., 2005). Several studies comparing traditionally used substrates (e.g. blood, faeces, saliva) to hair or examining the impact of stress-related conditions on hair cortisol concentration in both animal and human models provided evidence that hair is a suitable matrix for glucocorticoid analysis (Accorsi et al., 2008; Carlitz et al., 2014; González-de-la-Vara et al., 2011; Short et al., 2016; Tallo-Parra et al., 2015; Van Uum et al., 2008). Nevertheless, very low recovery of radio-labelled cortisol was reported in the hair of guinea pigs (*Cavia aperea f. porcellus*) (Keckeis et al., 2012) and non-systemic factors partly explained hair cortisol concentrations in brown bear (*Ursus arctos*) (Cattet et al., 2014). Further studies improving our understanding of hair as an indicator of HPA axis activity are thus needed (Cattet et al., 2017; Russell et al., 2012).

Glucocorticoid measurements using alternative biological matrices are increasingly used in ecological studies and can be especially useful for wild animal populations that are sensitive to invasive sampling (Buchanan and Goldsmith, 2004; Kersey and Dehnhard, 2014; Sheriff et al., 2011a). These alternative methods are, however, only suitable if native or metabolized glucocorticoid concentrations within the biological matrix reflect the physiological state of the animal (Sheriff et al., 2011a). This assumption must be validated for each new matrix and species using procedures such as an adrenocorticotrophic hormone (ACTH) challenge (Buchanan and Goldsmith, 2004; Sheriff et al., 2011a; Touma and Palme, 2005). With appropriate sample collection, faecal validations in captivity are usually successful and the expected peak in glucocorticoid metabolites is often detected several hours after the injection. Hair validation is especially difficult because the extensive integration time of this matrix makes it unlikely to reflect a single injection (Ashley et al., 2011). To our knowledge, a successful hair validation has not been published for non-domestic ungulates (but, see González-de-la-vara et al. (2011) and Ashley et al. (2011) for attempts on cattle and caribou, respectively).

The mountain goat (*Oreamnos americanus*) is an alpine ungulate especially sensitive to anthropogenic disturbance (Côté, 1996; Côté et al., 2013; Richard and Côté, 2016; White and Gregovich, 2017). Adverse effects of capture and chemical immobilization have been reported for this species, which exhibited life-history consequences following chemical

immobilization such as increased risk of kid abandonment and decreased kid production in young females (Côté et al., 1998). As with most mammals, cortisol is the dominant glucocorticoid in this species (Koren et al., 2012). Our objective was to validate the use of faecal glucocorticoid metabolite (FGM) and hair cortisol (HCC) concentrations as biomarkers of the HPA activity of the Rocky mountain goat. As such, we performed an ACTH challenge on captive animals using five weekly repeated injections. The effects of age, sex and reproductive status on glucocorticoid measurements were also assessed using faecal and hair samples collected from marked wild individuals during a long-term study. The influence of sample collection date (for faeces) and hair type (guard or undercoat) were also evaluated. Finally, we conducted a faecal degradation experiment to evaluate the impact of the time-lag between excretion and sample freezing on FGM measurements in alpine habitats.

1.4 Materials and methods

1.4.1 Animals

The ACTH challenge was conducted on captive mountain goats housed at the Calgary Zoo (Alberta). One adult male and 2 adult females were available for the faecal ACTH challenge. The same animals and a yearling female were used for the hair ACTH challenge. Both adult females had kids (~6 months old). Goats were fed a high-quality diet composed of a supplemented pelleted formula and mixed hay with *ad libidum* access to water. The habitat was divided into a large enclosure for exhibit, 2 pens where animals could be isolated, and 1 metal and polyethylene panelled restraint chute allowing handling without sedation. Goats were habituated to keepers entering the habitat on a daily basis and had been previously handled through the restraint chute.

1.4.2 Faecal ACTH challenge

The faecal ACTH challenge was conducted in late November 2017. Animals were used as their own control because of the low number of goats available. Females were isolated and challenged 1 day apart so that kids would not be left alone. The experiment was conducted

as follows: 1) One day before injection, we collected a baseline sample from every adult by entering the pen for less than half an hour and collecting spontaneously voided samples. The goats did not show signs of arousal such as quick movements or adopting a threatening position. 2) On the day of injection, goats were isolated into the restraint chute and we collected a second baseline sample either via spontaneous defecation or directly from the rectum. 3) Goats were administered 50 IU Synacthen Depot by intramuscular injection and kept isolated for a minimum of 20 hours. Based on studies in other ungulates, we expected faecal glucocorticoid metabolites to peak 12 to 20 hours post-injection of ACTH (Ganswindt et al., 2012; Gross et al., 1996; Kleinsasser et al., 2010; Miller et al., 1991; Spaan et al., 2017). We did not have access to the zoo facilities overnight. Therefore, we scheduled injections at 17:30 in order to produce a peak in glucocorticoid metabolites during the day, when we could precisely record excretion time. 4) We sampled all faeces voided after injection by collecting 10-20 pellets without visible urine contamination. First post-injection sample collection occurred at 07:30 (14 hours post-injection). We then visited the enclosures every 2 hours until the animals were released. For overnight samples collected in the morning or when more than one sample was excreted between two visits, we determined relative time of excretion based on the faeces physical characteristics (i.e. hot and moist samples considered more recent than dry and cold ones). Females were released 20 hours and 24 hours after injection according to the availability of the keepers. As a result of the unexpected low defecation rate in the adult male (only 1 post-injection sample after 20 hours), we maintained isolation for an additional 19 hours. Number of collected samples and post-injection isolation time were respectively 8 samples in 20 hours for the first female, 14 samples in 24 hours for the second female and 6 samples in 39 hours for the male. 5) We obtained group composite samples 5 and 6 days after the last injection by collecting overnight faeces in the pen where all animals were kept. Following the injection of the first female, we also opportunistically collected overnight samples from the pen where the other female (pre-injection) and 3 kids were kept. These group composite samples and all pre-injection samples were used to establish a baseline range of FGM concentration. All samples were stored at -20°C immediately after collection. The timetable of the experiment is presented by Figure 1.S1 in Supplementary material 1.8.1.

1.4.3 Hair ACTH challenge

The hair ACTH challenge was conducted from August to September 2016 during the period of active hair growth (Côté and Festa-Bianchet, 2003; Holroyd, 1967). Goats were guided and restrained in the chute. A small patch (3cm x 3cm) of rump hair was completely shaved using electric clippers and stored for analysis. Goats were administered 25 IU Synacthen Depot by intramuscular injection. Animals were then released in the enclosure and injections were repeated every 7 days for a total of 5 injections over 35 days. One week after the last injection, regrown hair was collected via shaving from the same patch initially shaved. Hair samples were stored in envelopes at room temperature until analysis.

1.4.4 Age, sex, reproductive status, date and hair type differences

We used 840 faecal samples and 580 hair samples (511 guard; 79 undercoat) collected on known individuals from 1990 to 2016 from a wild mountain goat population studied at Caw Ridge (54° 03' N, 119° 23' W), west-central Alberta, Canada. All goats included in the study were marked as juveniles and closely monitored every summer. Exact age, sex and reproductive status of each individual were therefore known for all samples. Details on the Caw Ridge study area and population are published elsewhere (Côté and Festa-Bianchet, 2001a, 2001b; Hamel et al., 2011).

All hair samples and most faecal samples (n=537) were collected upon capture. Hair samples were plucked from the rump and faeces were collected directly from the rectum of the goat. Both were frozen immediately after handling. Some faecal samples (n=303) were also collected opportunistically in the field from 2013 to 2016. We used a 50x camera mounted on a tripod to record the exact location and identity of individuals defecating and retrieved samples once the group had left (usually less than 30 minutes after excretion). Opportunistic samples were frozen within 2 to 6 hours post-excretion (mean freezing delay ± 95%CI of 175 samples for which defecation time was recorded in 2016 was 3.2 ± 0.3 hours). All faecal samples were stored in sterile individual bags and kept frozen in the dark until analysis to prevent any effect of UV light or changes in storage environment (e.g. humidity and temperature) on GC concentration. While other studies have shown that measurements in

hair are stable even after several months or years at room temperature (Macbeth et al., 2012, 2010; Yamanashi et al., 2016), hair samples were kept in the same storage condition as the faecal samples as an added precaution.

1.4.5 FGM degradation experiment

To assess the stability of FGM in alpine environments, we collected 5 faecal samples immediately after excretion. Each fresh sample was divided into 7 subsamples containing 6 pellets and treated as follows: one was immediately frozen (control), 3 were left outside on the ground exposed to wind and sun (sun group), and 3 were left outside placed in individual paper bags within a backpack in the shade (bag group). One subsample from each treatment group was later frozen after 4, 8 and 21 hours of exposure. The percent change in FGM concentration was calculated as the ratio between experimental subsamples and the associated control subsample. The experiment was conducted from 29 July 2017 at 11h19 to 1 August 2017 at 8h19. Shade temperature ranged from 14.6°C to 6.1°C and the sky was clear. We also measured temperature at 0, 4, 8 and 21 hours and report it along with the results.

1.4.6 Glucocorticoid extraction

Faecal samples collected in the wild were freeze-dried and crushed into powder to control for potential variability in water content associated with changing environmental conditions. The ACTH samples were collected from animals housed in a zoo under controlled conditions and were therefore extracted immediately post-thaw. Measurements from ‘wet’ and ‘dry’ samples are both valid and usually highly correlated, but absolute values vary because dried samples are more concentrated (Palme et al., 2013). To extract steroid hormones, we homogenised the samples and added 5 ml of 80% methanol to $0.20 \pm 0.01\text{g}$ of dry faeces or $0.50 \pm 0.01\text{g}$ of wet faeces. Immersed samples were left on a rotator plate overnight. After 22 hours, we centrifuged the samples to precipitate faecal material, transferred the supernatant into clean vials and stored them at -20°C until analysis. Prior to quantification by enzyme immunoassay (EIA), extracts were warmed to room temperature and diluted 1:20

in assay buffer (0.1mM sodium phosphate buffer, pH 7.0, containing 9g of NaCl and 1g of bovine serum albumin per litre).

Hair extractions were conducted in a laboratory and with tools that were never used for faecal or blood analyses. Hair samples were thoroughly examined for visible traces or smell of urine, faeces or blood and discarded if any signs of contamination were detected. Unstained samples were then sorted according to hair type and cut at their base to remove follicles. We cut hair in 5 mm pieces into pre-weighed 7 ml scintillation vials to obtain a sample mass (Mastromonaco et al., 2014). To avoid non-visible contamination with biological fluids, we washed all samples by vortexing them for 10s in 100% methanol and immediately removing all methanol with a pipettor (Mastromonaco et al., 2014). This wash procedure was designed to prevent any surface contamination without starting the extraction of GCs from the hair shaft. Immediately thereafter, we extracted steroid hormones by adding 80% methanol at a ratio of 0.01g of hair/ml of methanol, vortexing the vials for 5s and leaving them on a plate shaker for 24h (MBI Orbital Shaker; Montreal Biotechnologies Inc., Montreal, QC, Canada). We then centrifuged the samples at 2,400g during 10 min and pipetted the supernatant into new vials. Finally, extracts were air dried in a fume hood and stored at -20°C until analysis. Prior to quantification by EIA, dried extracts were warmed to room temperature and reconstituted in assay buffer for one hour with vortexing at the beginning and end of reconstitution. Due to the low GC concentration present in hair, dried extracts were reconstituted as a 10-fold concentration of the original extracts.

1.4.7 HPLC Analysis

Prior to immunoassay, characterization of steroid metabolites in the faecal extracts was done using reverse phase high performance liquid chromatography (HPLC). Preparation of the samples for HPLC was done by evaporating 25 ml of faecal extracts from a pool of 5 samples. The preparation was then purified by solid-phase extraction using Sep-Pak C₁₈ cartridges (Waters Scientific, Mississauga, ON, Canada) and analysed using a Waters Alliance 2695 HPLC separation system as previously described by Kummrow et al., 2011. Cortisol and corticosterone (Steraloids Inc.) were run as reference standards. Absorption was recorded at 254 nm. Eluent fractions were collected every minute for 20 minutes and then assayed with

both cortisol and corticosterone EIA to determine immunoreactivity. Based on immunoreactivity peak, we selected the cortisol EIA for all further analyses (see results).

1.4.8 Immunoassay

FGM and HCC were assayed by EIA using the cortisol antiserum R4866 (raised against cortisol-3CMO-BSA; C. Munro, University of California, Davis, CA, USA) and cortisol-HRP conjugate (C. Munro, University of California, Davis, CA, USA) following the method previously described by Majchrzak et al. (2015). In brief, microtitre plates (Nunc maxisorp, VWR, Mississauga, ON, Canada) were coated with cortisol antiserum diluted 1:12,000 in coating buffer overnight at 4°C. Faecal extracts were diluted 1:20 in EIA buffer and evaporated hair extracts were reconstituted in EIA buffer at a 10X concentration. Samples, cortisol standards (78-20,000 pg/ml; Sigma H-0135), and controls were loaded along with horseradish peroxidase conjugate (diluted 1:34,000 in EIA buffer) onto the plate and incubated for 2 h at room temperature. The substrate solution (ABTS) was then added and absorbance was measured at 405 nm using a spectrophotometer (MRX microplate reader, Dynex Technologies, Chantilly, VA, USA). The cross-reactivities of the cortisol antiserum were: cortisol, 100%; prednisolone, 9.9%; prednisone, 6.3%; cortisone, 5%; corticosterone, 0.7%; 21-deoxycortisone, 0.5%; deoxycorticosterone, 0.3%; other, <0.3% (Young et al., 2001). Assay sensitivity was 41.8 pg/mL. Biochemical validation (parallelism and recovery) of the cortisol EIA was done using pooled faecal and hair extracts. Inter-assay CV's were <15% and intra-assay CV's were <6% for both assays and sample matrices.

1.4.9 Statistical analysis

We defined the baseline range for the faecal ACTH challenge as the 95% confidence interval (hereafter CI) of average FGM concentration using all samples collected prior to and over 5 days after injection. FGM concentrations outside this baseline range were considered elevated. We compared the highest FGM concentration of each injected animal with the mean baseline concentration using a one-sided t-test. We also report the relative increase between pre-injection and maximum concentrations. For the hair ACTH challenge, we conducted a

one-sided paired t-test to compare pre- to post- injection HCC while accounting for individual variation. Relative increase in HCC are also reported.

We assessed the impact of collection date on FGM and hair type on HCC using samples collected from the wild population of Caw Ridge. For faecal samples, we compared a set of candidate linear mixed models using Akaike information criterion (AIC_c ; Burnham and Anderson, 2002). The base model included age class (kid, yearling, 2-years-old or adult) and sex as fixed covariates, and year and goat identity as random factors, while other candidate models also included Julian date of collection fitted either as 1st, 2nd, 3rd or 4th polynomial order.

For hair, we used 50 samples containing both guard and undercoat hair and compared cortisol concentration of hair types with a paired t-test using the sample ID as grouping factor. We also conducted an analysis of variance to compare different hair samples ($n=598$) from the Caw Ridge population that were sorted as guard or undercoat before extraction. Finally, we tested the correlation between guard and undercoat HCC with a linear model. One highly influential value (Cook's distance: 9.17) was excluded when fitting the model, but the outcome of the test was the same with or without this value.

We built a set of candidate linear mixed models to test the effects of age class, sex, reproductive status (lactating/non-lactating) and the interaction between age class and sex on FGM and HCC, and selected the most parsimonious model using AIC_c . The base model included goat identity and year fitted as random factors. Other candidate models also included age class, sex or both age class and sex. We conducted a second model selection based exclusively on adult females and using the same random factors (identity and year) to evaluate the influence of reproductive status. Date of sample collection was included as a fixed covariate (second order polynomial term) in all FGM models to account for its impact on FGM concentration (see results, subsection 1.5.4). Only guard hair samples were used in the hair analyses because of the effect of hair type on HCC (see results, subsection 1.5.4). Because mountain goat hair grow between May and early December (Côté and Festabianchet, 2003), fully grown hair collected during summer were used to evaluate the physiological status of the previous year.

We built a linear mixed model with freezing delay (0, 4, 8 and 21 hours) and treatments (sun/bag/none) as fixed effects and sample identity as random factor to assess the impact of congelation delay on FGM concentration in alpine environments. We report parameter estimates and 95% confidence interval.

All statistical analyses were conducted with R 3.4.3 (R Core Team, 2017). We used the package “lme4” (Bates et al., 2015) to conduct linear mixed models and “AICcmodavg” (Mazerolle, 2017) for model selection. Normality and homoscedasticity were inspected visually through the distribution of residuals and residuals-fitted plots. To meet these assumptions, FGM data were ln-transformed for all analyses except the ACTH challenge and the degradation experiment whose residuals were normally distributed. For simple linear regression, analysis of variance and t-test, we report p-values with a significance threshold of $\alpha=0.05$. For AIC_c selection, we present 95% CI for all parameters included in plausible models ($\Delta\text{AIC}_c < 2$) but considered the most parsimonious model as the best one (Burnham and Anderson, 2002). For all mixed models, we calculated r^2 using MuMin package (Barton, 2016) and present the marginal and conditional r^2 as defined by Nakagawa and Schielzeth (2013). For linear mixed models with categorical variables, we conducted multiple comparisons under a model selection framework using *multComp* function of the package “AICcmodavg” (Mazerolle, 2017).

1.5 Results

1.5.1 Validity of immunoassay

Biochemical validations showed that the cortisol assay was suitable for faeces and hair. The recoveries of known concentrations of exogenous cortisol from faecal and hair extracts were $111 \pm 4\%$ and $104 \pm 5\%$, respectively. The measured hormone concentrations in the spiked samples correlated with the expected concentrations (faecal cortisol $r=0.999$, $p< 0.001$; hair cortisol $r=0.996$, $p< 0.001$; Figure 1.S2). Serial dilutions of pooled faecal and hair extracts showed parallel displacement with the cortisol standard curve (faecal cortisol $r=0.994$, $p< 0.001$; hair cortisol $r=0.987$, $p< 0.001$; Figure 1.S3).

Chromatographic separation of pooled faecal extracts resulted in several peaks at fractions near the cortisol reference standard and no major peak thereafter (Figure 1.1A). Immunoreactivity of the eluted fractions was evaluated using both cortisol and corticosterone EIAs. The cortisol EIA produced one major peak, which corresponded to the fraction that eluted at the same time as the cortisol standard on the chromatogram (Figure 1.1). The corticosterone EIA did not produce any significant peaks, but smaller peaks that did not correspond to the elution time of either cortisol or corticosterone standards on the chromatogram (Figure 1.1). These results determined the selection of the cortisol EIA for all further analyses.

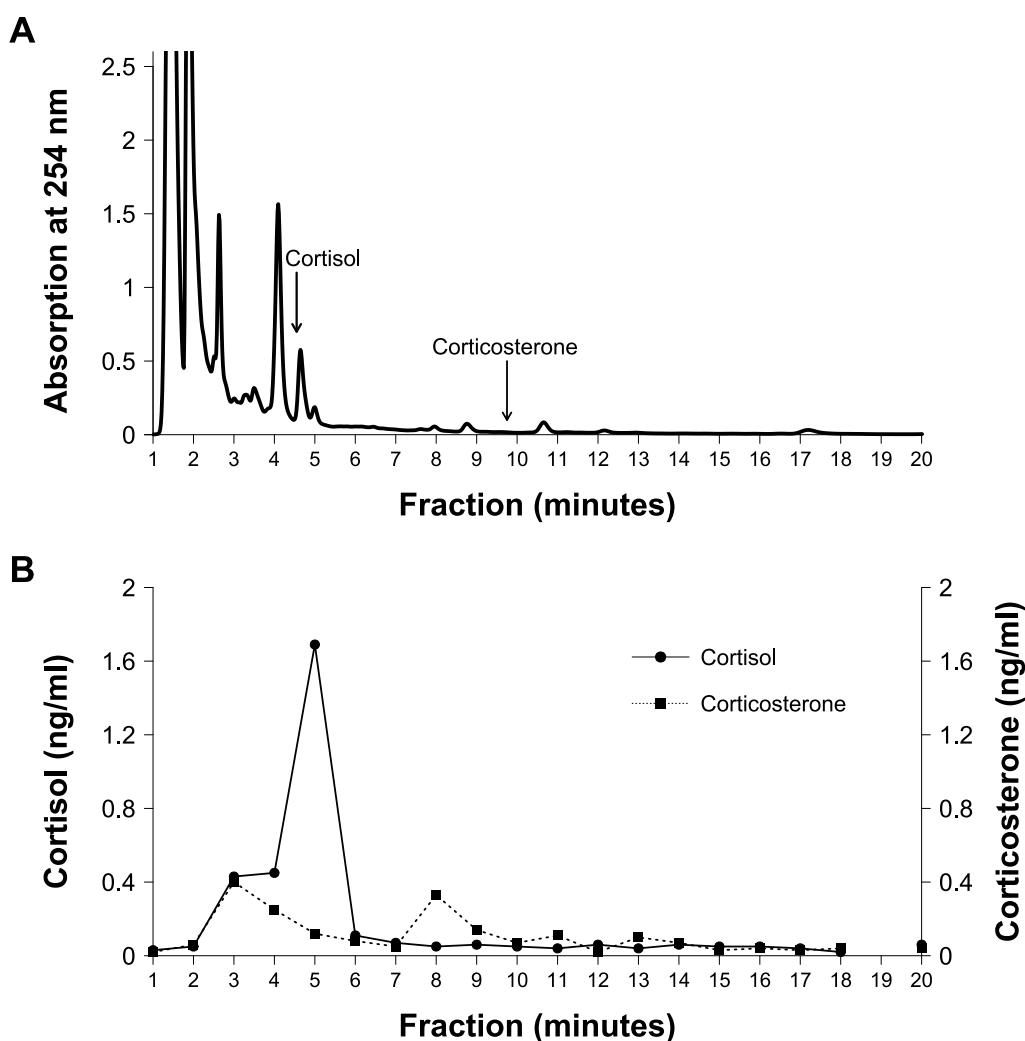


Figure 1.1 Reverse phase high performance liquid chromatography (HPLC) separation of pooled faecal extracts from captive mountain goats: (A) chromatogram and (B) immunoreactivity profile as determined by cortisol and corticosterone enzyme immunoassay.

1.5.2 ACTH challenge

The faecal ACTH challenge revealed a clear rise in FGM concentrations for two of the three injected animals and a moderate rise for the third one (Figure 1.2). The adult male and one adult female reached maximum FGM concentrations 32 hours (192% of baseline concentration) and 21 hours (223%) post-injection, respectively. Overnight samples from the other adult female collected the morning after injection were elevated (127%), but maximum concentration (131%) occurred 20 hours after injection in the last sample. Peak FGM concentrations were significantly higher than the baseline average for all animals (Adult female 1: $t_{12}=-18.5$, Adult female 2: $t_{12}=-6.0$, Adult male: $t_{12}=-7.6$; All p's <0.0001).

For the hair ACTH challenge, there was a clear rise in HCC following repeated ACTH injections ($t_3=-5.4$, p=0.006; Figure 1.3). Relative change in cortisol concentrations for the two adult females, the yearling female and the adult male were respectively 264%, 147%, 240% and 233%.

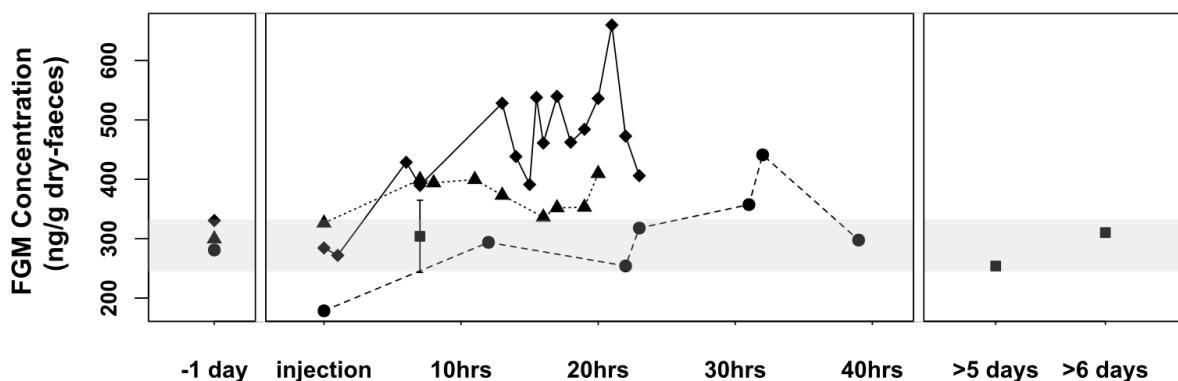


Figure 1.2 Faecal glucocorticoid metabolite (FGM) concentrations of two adult females (diamond and triangle) and one adult male (circle) mountain goat following one adrenocorticotrophic hormone (ACTH) injection. Black squares are baseline group composite samples. Shaded grey area represents 95% confidence interval of the average baseline concentration.

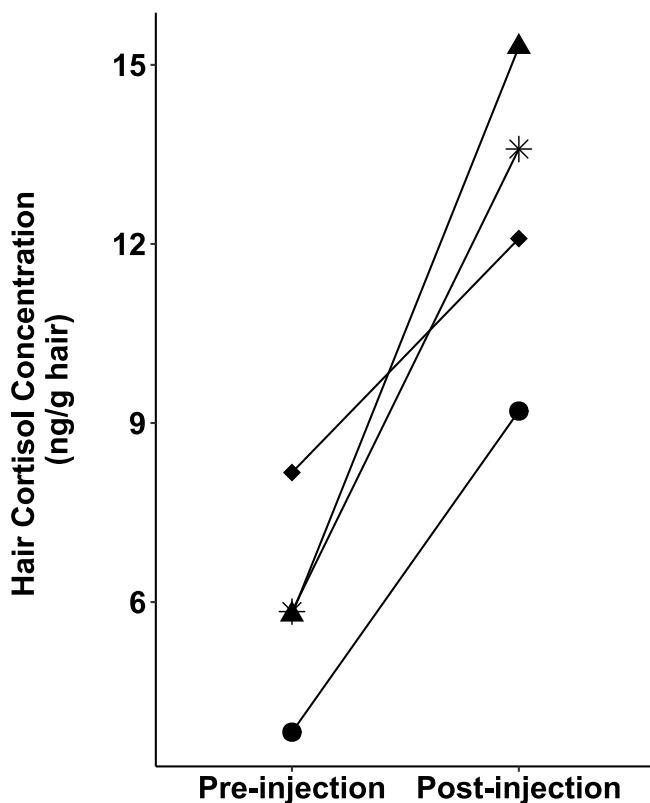


Figure 1.3 Hair cortisol concentrations (HCC) of two adult females (diamond and triangle), one yearling female (asterisk) and one adult male (circle) mountain goat before and after a trial of 5 repeated adrenocorticotropic hormone (ACTH) injections.

1.5.3 Faecal sample collection date and hair type

The best model assessing the effect of collection date on FGM concentrations included Julian date as a second order polynomial term ($R^2_m = 0.07$, $R^2_c = 0.38$; Table 1.S1 in Supplementary material). The model with Julian date as a fourth order polynomial term had equivalent support but was less parsimonious ($R^2_m = 0.07$, $R^2_c = 0.39$; Table 1.S1 in Supplementary material). Average FGM concentrations increased until mid-July and decreased afterwards (ln-scaled estimate [95% CI]: Day -0.14 [-1.21, 0.94], Day² -3.64[-4.51, -2.77]; Figure 1.4). Effect size was large with average cortisol concentration in June and July being 1.5 times higher than in May and 2 times higher than in September (FGM[95%CI] average: May 355[294,417], June 563[534,593], July 545[500,591], Aug. 459[434,485], Sept. 248[204,292]).

Hair cortisol concentration was highly related to hair type for guard and undercoat hair belonging to the same sample ($t_{51}=11.7$, $p<0.001$). Guard hair had on average 2.35 times more cortisol than undercoat hair. The same pattern was observed when comparing all

available hair samples taken on different individuals at Caw Ridge (mean \pm SE: Guard 10.4 \pm 0.2, Undercoat 7.2 \pm 0.1; $F_{1,596}=51.9$, $p<0.001$). Samples for which both guard and undercoat hair were analysed revealed a highly significant but weak correlation of cortisol concentration among hair types (estimate \pm SE: 0.98 \pm 0.23, $F_{1,49}=18.2$, $p<0.001$, $R^2 = 0.27$; Figure 1.5).

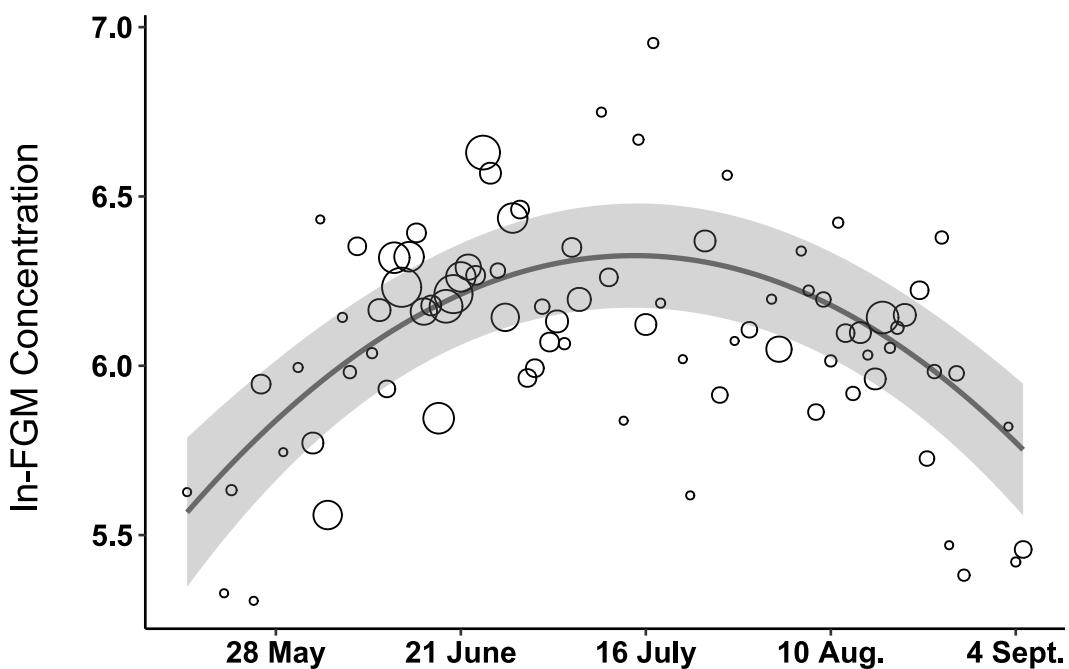


Figure 1.4 Faecal glucocorticoid metabolite (FGM) concentration during summer in mountain goats from the Caw Ridge population (Alberta) sampled between 2000 and 2016. Dot size is proportional to sample size. The fitted quadratic regression is presented with its 95% confidence interval and includes age class and sex as fixed covariates and goat identity and year as random factors.

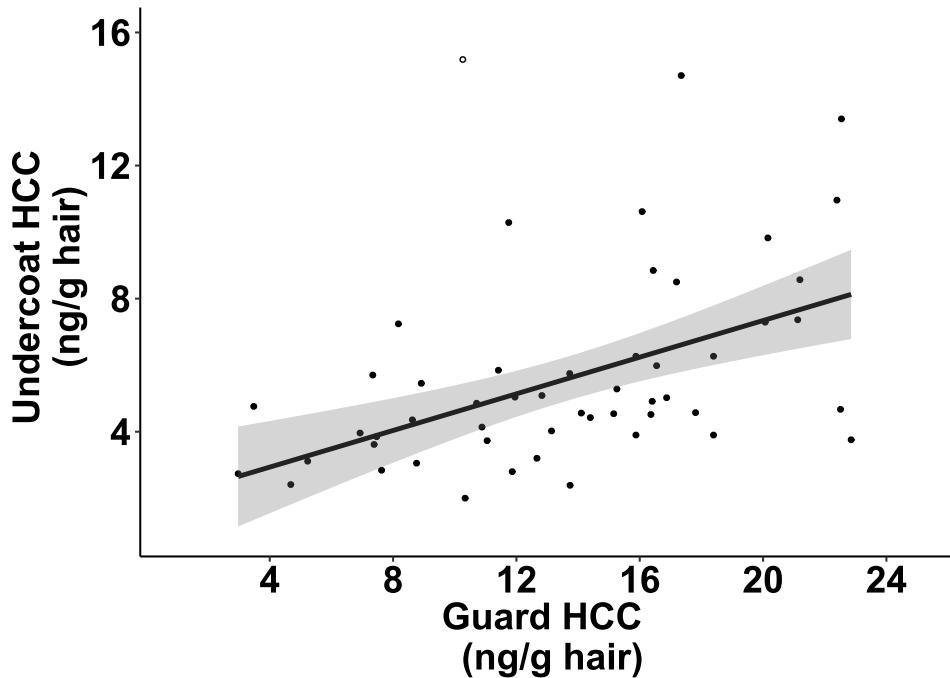


Figure 1.5 Correlation between hair cortisol concentrations (HCC) of guard and undercoat hair in mountain goats, at Caw Ridge, Alberta (2000-2016) from 50 samples that contained both hair types. Linear regression is presented with 95% confidence interval and was fitted without the influential point represented by an empty circle (Cook's distance 9.17).

1.5.4 Age, sex and reproductive status

Age class and sex do not appear to have a strong influence on mountain goat FGM as these variables were not included in the best model (Table 1.1). The model that included sex alone had equivalent support as the base model, but was less parsimonious and the estimate's confidence interval overlapped 0 (In-scaled estimate[95%CI]: 0.02[-0.04,0.09]). The model with the lowest ΔAIC_c for hair included the interaction between age class and sex (Table 1.1, $R^2_m = 0.10$, $R^2_c = 0.36$). According to this model, males had lower HCC than females, but only when adults (estimate[95%CI]: Yearling:Male -0.68[-2.02,0.65], 2-years-old:Male 0.24[-2.33,2.82], Adult:Male -1.83[-3.41,-0.26]). However, the model including age class alone had equivalent support (Table 1.1) and was more parsimonious (Table 1.1; $R^2_m = 0.09$, $R^2_c = 0.34$; estimate[95%CI]: Yearling 0.03[-0.65,0.70], 2-years-old -2.06[-3.08,-1.04], Adult -2.47[-3.22,-1.71]). Multiple comparisons revealed that kids and yearlings had higher HCC than 2-years-olds and adults (Figure 1.6; Table 1.S2 in Supplementary material). Reproductive status of females did not appear to influence FGM or HCC as the base models

had equivalent support and were more parsimonious (Table 1.1). Both FGM and HCC seemed marginally lower in barren females, but the confidence intervals of these estimates included 0 (ln-FGM: -0.1[-0.24,0.04]; HCC: -0.54[-1.98,0.90]).

Table 1.1 Model selection for the influence of age class, sex and reproductive status on faecal glucocorticoid metabolite concentration and hair cortisol concentration in mountain goats, at Caw Ridge, Alberta (2000-2016). First set of candidate models (a) is based on the full data set (nfaeces=761; nhair=511), second set (b) is based on a subset of adult females for which reproductive status was known (nfaeces=271; nhair=115). Individual identity and year were included in all models as random factors. K is the number of parameters. AICc wt is the relative weight of the model compared to other candidate models.

Models	Faecal Metabolite Concentration				Hair Cortisol Concentration			
	K	AICc	ΔAICc	AICc wt	K	AICc	ΔAICc	AICc wt
a)								
Base	6	793.0	0	0.59	4	2732.8	50.7	0
Age	8	795.9	3.0	0.13	7	2682.6	0.5	0.44
Sex	7	795.6	1.6	0.27	5	2724.3	43.2	0
Age + Sex +								
Age:Sex	11	801.5	8.6	0.01	11	2681.1	0	0.56
b)								
Base	6	262.6	0	0.54	4	623.8	0	0.70
Reproductive status	7	262.9	0.3	0.46	5	624.5	1.7	0.30

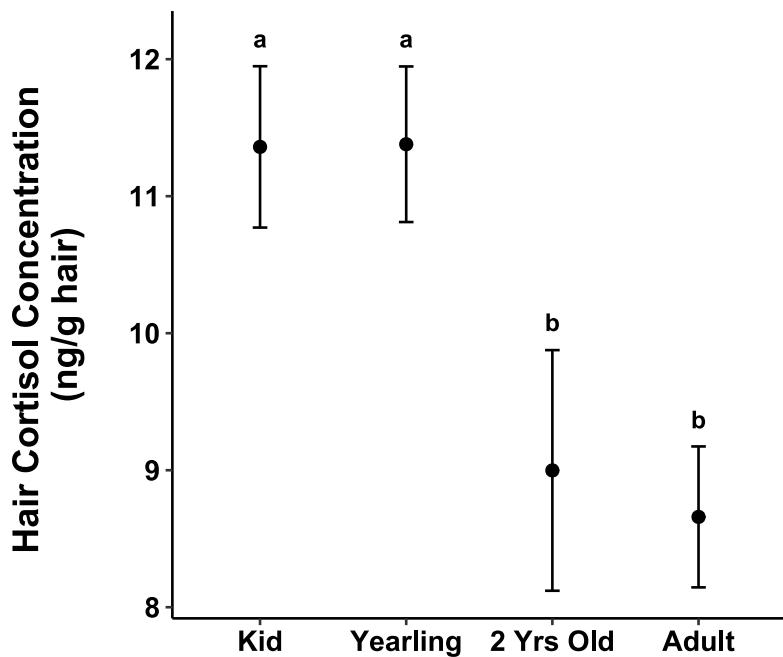


Figure 1.6 Average hair cortisol concentrations according to age-classes in mountain goats, at Caw Ridge, Alberta (2000-2016). Error bars represent 95% confidence intervals, letters indicate grouping structure determined by multiple comparisons.

1.5.5 Faecal metabolites degradation

The degradation experiment did not reveal clear directional variation of FGM concentration for samples exposed to sun (4 hours: 0.2[-11.2,11.6], 8hours: -3.3[-14.7,8.0], 21 hours: 4.9[-6.5,16.2]; Figure 1.7). Average concentrations of samples kept in a bag were consistently lower than concentrations of controls, but effect size were similar regardless of the duration of the freezing delay (4 hours:-8.3[-18.6,2.0], 8 hours: -11.1[-21.4,-0.9], 21hours: -7.6[-17.9,2.7]; Figure 1.7). FGM concentration varied widely between controls and treatment subsamples with a total range of -19.9% to 19.4% for sun exposed samples and -28.2% to 4.8% for samples kept in a bag.

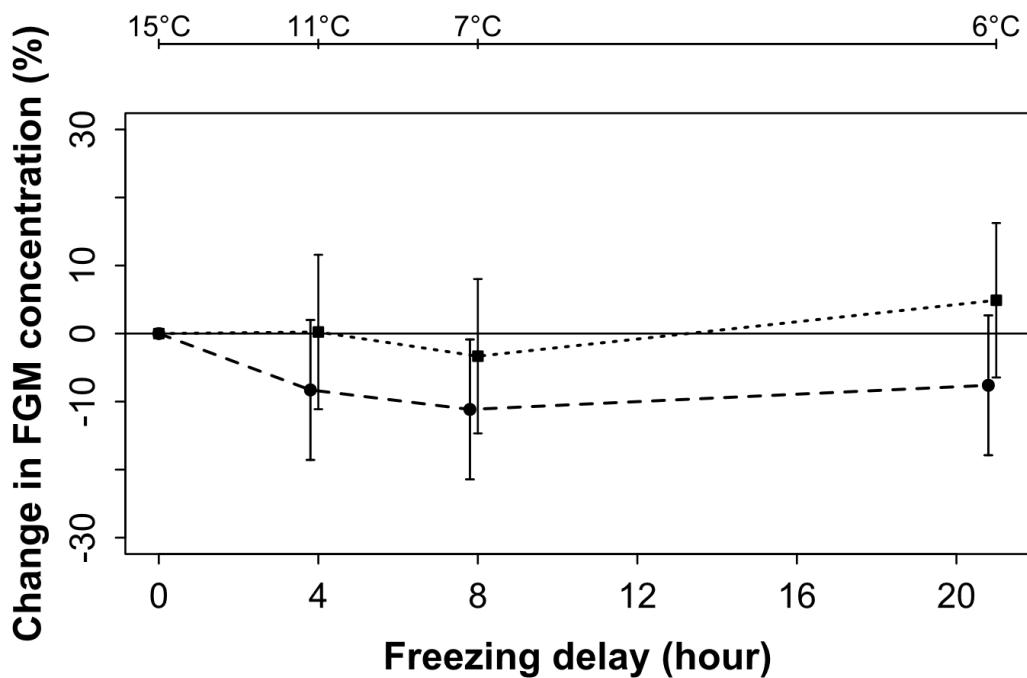


Figure 1.7 Variation of FGM concentration in mountain goats, at Caw Ridge, Alberta (2000-2016), after 0, 4, 8 and 21 hours of exposure to environmental conditions either in the sun (square) or in a bag (circle). Error bars represent 95% confidence intervals. Ambient shade temperature recorded at 0, 4, 8 and 21 hours is shown above graph.

1.6 Discussion

We validated a method to measure HPA axis activity in mountain goats using faecal and hair samples. We showed that a cortisol EIA can be used to reliably monitor FGM and HCC in this species. To our knowledge, this is also the first demonstration that systemic HPA axis activity is reflected in the hair of a non-domestic ungulate.

1.6.1 Validity of immunoassay

EIA performance was validated with parallelism, accuracy and precision tests, which showed that the assay could reliably measure the hormones present in the faecal and hair extracts without confounding effects of factors within the extract. Characterization of the faecal hormone metabolites by HPLC revealed that the immunoreactive substances present in our

extracts were similar to the cortisol standard in terms of polarity and that the cortisol-EIA was best suited to measure them. Previous studies have shown that native cortisol is often virtually absent in the faeces of mammals (reviewed in Palme et al., 2005, but see Goncalves et al., 2016 and Heistermann et al., 2006). Therefore, it is unclear if the assay measured authentic cortisol or cross reacted with metabolized forms of similar polarity (Möstl et al., 2005). Interestingly, no significant peak was detected on the chromatogram or the immunogram over 7 minutes, suggesting that less polar metabolites typically observed in ruminant feces (11,17-dioxoandrostanes; Palme and Mostl, 1997) were not present in our extracts. Since the HPLC fractions were collected every minute, we do not know if the major immunoreactive peak observed in fraction 5 was caused by the compounds identified by the chromatogram at approximately 4.1 minutes, 4.6 minutes, or by both. However, irrespective of the specific identity of the substance being measured, the detection of the ACTH injection by the cortisol-EIA demonstrates that this assay quantifies faecal metabolites in a biologically-relevant manner.

1.6.2 ACTH challenge

Administration of ACTH induced a clear response in FGM for the male and one female, but the excretion pattern for the second female was not as obvious. Because we expected a shorter delay between injection and excretion of immunoreactive substances, this animal was released 20 hours post-injection whereas peak concentration was observed after 21 hours in the other female. It is therefore unclear whether FGM concentration continued to rise or decreased after it was released in the exhibit. Nevertheless, post-injection samples were elevated compared to baseline samples indicating that this female reacted to ACTH but might not have been kept long enough to measure the decrease in FGM concentration. Highest FGM concentrations were detected 20 to 32 hours post-injection. (dairy goat (*Capra aegagrus*) 13h (Kleinsasser et al., 2010), red deer (*Cervus elaphus*) 18.75h (Huber et al., 2003b), African buffalo (*Syncerus caffer*) 10-20h (Spaan et al., 2017), sheep (*Ovis aries*) 12h and horse (*Equus caballus*) 24h (Palme et al., 1996)). Comparing horses, sheep and pigs (*Sus domesticus*), Palme et al. (1996) showed that the time course of steroid excretion in the faeces was highly related to gut passage time. Gut passage time decreases when an animal is active (Touma et al., 2003). Delay between injection of ACTH and peak FGM concentration should

therefore be longer when injection occurs at a time of low activity (Touma et al., 2003). Mountain goats are diurnal and animals in our study were injected at sunset which could explain the slightly longer delay we observed. While it is unclear why the male had such a low defecation rate (only 1 faecal output after 20 hours compared to 8 and 11 for the females), this is likely the cause of the much later peak observed for this individual. Variability in the time course of excretion should be kept in mind when interpreting FGM measurements from wild mountain goats especially when the impact of short-term stressors is assessed.

The hair ACTH challenge resulted in an unequivocal rise of HCC for all animals after injection. This clearly demonstrates that systemic fluctuations of cortisol can be reliably monitored in this substrate. However, while relative elevation was similar across individuals ($221\pm26\%$), there was substantial variability in absolute CORT concentration. Indeed, after a 233% rise in cortisol concentration, the male peak HCC was similar to the baseline HCC of one female. Using single samples without knowing an animal baseline level could therefore be misleading. It should also be kept in mind that the goats in our study were in ideal conditions avoiding excessive abrasion on the skin or other sources of local stress that could artificially elevate HCC (Salaberger et al., 2016).

1.6.3 Faecal sample collection date and hair type

The hormonal pattern observed for the effect of collection date, with highest FGM levels occurring in mid-summer, is consistent with seasonal FGM variation in elk (*Cervus canadensis*; Millspaugh et al., 2001) and bighorn sheep (*Ovis canadensis*; Goldstein et al., 2005), but opposite to Pyrenean chamois (*Rupicapra pyrenaica pyrenaica*: Dalmau and Manteca, 2007) and red deer (Huber et al., 2003). Annual variation of GC concentration can be the result of various factors including changes in anthropogenic activity (Millspaugh et al., 2001), altered gut passage time due to changes in forage quality (Hofmann, 1989) and changes in life-history stages such as lactation (Landys et al., 2006; Romero, 2002). All-terrain vehicles (ATVs) have access to Caw Ridge and their presence usually increases after mid-June, but a previous study suggested they were not perceived as a major threat by the goats (St-Louis et al., 2013) and large groups of ATVs can be seen in early September when average FGM concentration was at the lowest. Increased GC concentration due to longer gut

passage time is also unlikely since forage quality peaks in mid-summer and should therefore shorten gut passage time. Finally, elevation of GC concentration could help females sustain lactation (Landys et al., 2006), but this does not explain a similar pattern in males and juveniles. Our sample size in early and late summer was low compared to mid summer. Further analyses with standardized sample collection across summer would help understand the drivers of the observed variation in FGM. Nonetheless, we advise to take seasonal patterns into account as not doing so could bias the interpretation of FGM results.

Hair cortisol concentration was much lower in undercoat than guard hair. Hair type was a strong predictor of HCC even when pooling all samples taken between years 2000 and 2016 from individuals likely exposed to different stressors. This result is consistent with a previous study by Macbeth et al. (2010) which showed that guard hair contains more cortisol than undercoat hair in brown bear. Hormone incorporation can vary according to chemical and physical characteristics of hair types (Harkey, 1993; Pragst and Balikova, 2006). Another possibility is that guard and undercoat hair growth period do not perfectly overlap as seen in other ungulates (Cowan and Raddi, 1972; Nixon et al., 1991). Both hair types would therefore provide distinct but equally valid information on cortisol exposure. The systematic differences we observed could for instance be explained by seasonal variation in cortisol levels. Cortisol concentrations were significantly correlated when analysing both hair types from the same sample. However, the predictive power of the correlation was low and would likely yield imprecise estimates if it was used to account for the effect of hair type in mixed data sets. It is important to note that all samples in our study were collected from the rump to standardize for any differences in HCC due to body location (Ashley et al., 2011; Macbeth et al., 2010; Terwissen et al., 2013). Our results support those of Macbeth et al. (2010) and strengthen the idea that comparative analysis should be restricted to one hair type.

1.6.4 Age, sex and reproductive status

Age class and sex had no detectable impact on mountain goat FGM concentration in our study. While sex-specific differences in FGM excretion have been found in various taxa including rodents and birds (Goymann, 2012; Palme et al., 2005), no effect of sex on FGM measurements were detected in other wild ungulates such as red deer (Huber et al., 2003a),

chamois (Hadinger et al., 2015) and elk (Millspaugh et al., 2001). Aging has been shown to influence systemic glucocorticoid concentration through alteration of the HPA-axis responsiveness in rodents (e.g. Sapolsky, 1991) and primates (e.g. Goncharova and Lapin, 2002). Pavitt et al. (2015; 2016) showed that red deer also have higher FGM concentration as they age and attributed this to a decline in body condition. Our results suggest that age and sex have limited influence on mountain goat FGM concentration or that other environmental factors are more influential under natural conditions.

Age class and, to a lesser extent, sex influenced hair cortisol concentrations. Kids and yearlings had higher HCC than older individuals, and females had higher HCC than males but only when adult. Plasma cortisol concentration can vary according to age and sex (Touma and Palme, 2005), but molt speed and hair morphology may also influence cortisol incorporation into hair (Henderson, 1993). The age- and sex- specific differences observed in our study match the differences in molt timing observed by Déry et al. (2019) on the same population. Indeed, according to this study, yearlings and adult females complete the shedding of their winter coat later than older individuals and males, likely because of variation in energetic constraints and access to nutritional resources (Déry et al., 2019). The age- and sex- specific differences we observed could therefore either reflect real changes in systemic HPA-axis activity potentially also linked to energy management or represent an artefact of molting patterns. Nevertheless, the clear change in HCC after 2 years and the potential sex difference for adults underline the importance of individual identification when using hair samples to assess cortisol concentration. The use of non-invasive sampling methods such as barbed-wire hair traps should be coupled to other methods allowing age- and sex- identification (e.g. DNA analysis, camera).

We did not detect a difference in FGM and HCC between lactating and non-lactating females. Female glucocorticoid concentration is elevated during lactation in most mammalian species (Edwards and Boonstra, 2018). However, even toed ungulates such as sheep, cows (*Bos taurus*) and red deer do not show this pattern (Edwards and Boonstra, 2018). Our results in mountain goats are therefore consistent with other similar species.

1.6.5 Faecal metabolites degradation

Prolonged exposure to ambient temperature reduced the immunoreactive substances measured by our assay for subsamples kept in a bag but not for subsamples exposed to sun. Cortisol metabolites can be further metabolized by bacterial enzymes following defecation (Möstl et al., 2002). FGM concentrations measured by an immunoassay will then either increase, decrease or remain stable depending upon the assay sensitivity to the altered metabolites (Lexen et al., 2008; Ludwig et al., 2013; Möstl et al., 2002). Bacterial activity might have been facilitated by the relative humidity of the bag but limited in faeces dried after sun exposure. Interestingly, subsamples exposed for 4, 8 and 21 hours had similar FGM concentrations suggesting that bacterial degradation occurred in the first hours of exposure. Ambient temperature decreased during the experiment and might have limited further bacterial activity. The relative FGM concentration varied widely between subsamples. Because we wanted our experiment to reflect real exposure conditions, we avoided breaking structural integrity of the pellets and did not homogenize the samples prior to separating them. Some of the variability we observed is thus likely attributable to intra-sample variation (Millspaugh and Washburn, 2003). Our results suggest that glucocorticoid metabolite degradation can occur in mountain goat faeces exposed to environmental conditions. However, degradation seemed limited by exposure to wind and sun or by low ambient temperature.

This study demonstrates that glucocorticoid concentrations can be successfully measured in both faeces and hair of mountain goats using a cortisol enzyme immunoassay. Metabolites in the faeces are representative of GC concentrations one or two days prior to sample collection and hair can reflect multiple weeks of glucocorticoid exposure. To avoid biased measurements, faecal samples should be kept at low temperature and frozen as soon as possible. Age class, sex and reproductive status had no detectable effect on FGM measurements, but date of sample collection must be considered as it could impact interpretation. On the other hand, hair cortisol concentration of different age- and sex- classes should be analysed separately and comparative analysis should be restricted to one hair type.

1.7 Acknowledgements

This study was primarily funded by the Natural Sciences and Engineering Research Council of Canada. We obtained additional financial or logistical support from the Alberta Fish and Wildlife Division and the Alberta Conservation Association. F.D.B. received a scholarship from the Fonds de Recherche du Québec sur la Nature et les Technologies. We are grateful to F. Déry, B. Carrier, G. Carenini and the many people who helped with fieldwork over the years at Caw Ridge. We thank M.C. Martin for her tremendous contribution to the laboratory work at Laval University as well as C. Gilman and S. Matteer for all their help with the enzyme immunoassay and the biochemical validations. We also thank N. Bouwman, M. Imfeld, G. Misurelli, L. Palmer, and S. Russell for their help with the ACTH challenge and B. Peters for her generous assistance and logistic support in Calgary. We are thankful to S. Hamel, A. Langwieder, F. Letourneau, O. Love, J.H. Richard and two anonymous reviewers for their constructive comments that greatly improved this article.

1.8 Supplementary material

1.8.1 ACTH challenge timetable

Time	Monday 27 Nov.	Tuesday 28 Nov.	Wednesday 29 Nov.	Thursday 30 Nov.	Monday 4 Dec.	Tuesday 5 Dec.
07:00 - 08:00			Overnight sample collection (♀1 & ♂). Additional opportunistic sample collection from the pen where ♀2 (not yet injected) and the kids were kept.	Overnight sample collection (♀2 & ♂) ♂ is released.		
08:00 - 09:00						
09:00 - 10:00						
10:00 - 11:00			Sample collection every 2 hours (♀1 & ♂)			
11:00 - 12:00						
12:00 - 13:00	Collection of baseline samples (♀1, ♀2, ♂)		♀1 is released. Prolongation of sample collection for the ♂ (only 1 sample post-injection by that time).	Sample collection every 2 hours (♀2)	Group composite sample collection (overnight) from the pen where all animals were kept.	Group composite sample collection (overnight) from the pen where all animals were kept.
13:00 - 14:00				♀2 is released.		
14:00 - 15:00			Isolation, collection of baseline samples and Injection (♀2). Continued sample collection (♂)			
15:00 - 16:00						
16:00 - 17:00		Isolation and Collection of baseline samples (♀1 & ♂)	Sample collection every 2 hours (♀2 & ♂)			
17:00 - 18:00		Injection (♀1 & ♂)				

Figure 1.S1 Schedule for the faecal ACTH challenge conducted on two adult females and one adult male mountain goat at the Calgary zoo from 27 November to 5 December 2017.

1.8.2 AIC table for the effect of faecal sample collection date

Table 1.S1 Model selection for the influence of date of collection on faecal glucocorticoid metabolite concentration in mountain goats, at Caw Ridge, Alberta (2000-2016). All models included age class and sex as fixed covariates and goat identity and year as random factors. K is the number of parameters.

Models	K	AICc	ΔAICc	AICc weight
Base + Day + Day ² + Day ³ + Day ⁴	12	806.8	0	0.54
Base + Day + Day²	10	807.9	1.2	0.31
Base + Day + Day ² + Day ³	11	809.4	2.6	0.15
Base	8	869.0	62.2	0
Base + Day	9	870.8	64.0	0

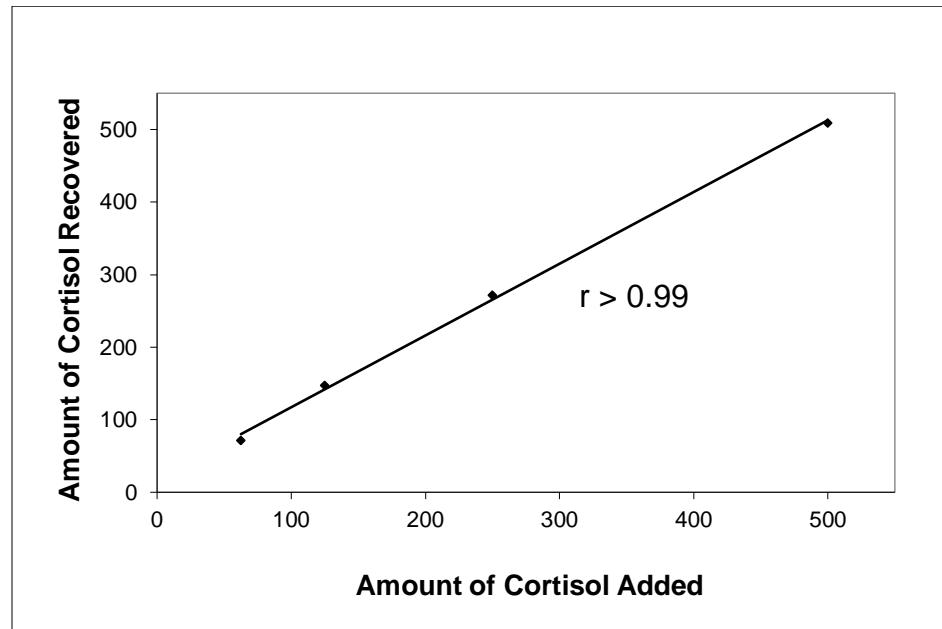
1.8.3 Grouping patterns for the effect of age class

Table 1.S2 Multiple comparisons of grouping patterns for the effect of age class on hair cortisol concentration in mountain goats, at Caw Ridge, Alberta (2000-2016), using model selection with Akaike information criterion corrected for small sample size (AICc). K is the number of parameters.

Grouping structure				K	AICc	Δ AICc	AICc weight
Adult	2-yrs-old	Yearling	Kid				
a	a	b	b	5	2678.1	0	0.50
a	b	c	c	6	2679.6	1.5	0.24
a	a	c	b	6	2680.1	2.0	0.18
a	b	d	c	7	2681.6	3.5	0.08
a	b	b	b	5	2694.8	16.7	0
a	b	c	b	6	2694.9	16.8	0
a	a	b	a	5	2720.5	42.5	0
a	a	a	a	4	2731.8	53.7	0

1.8.4 Biochemical validations

a)



b)

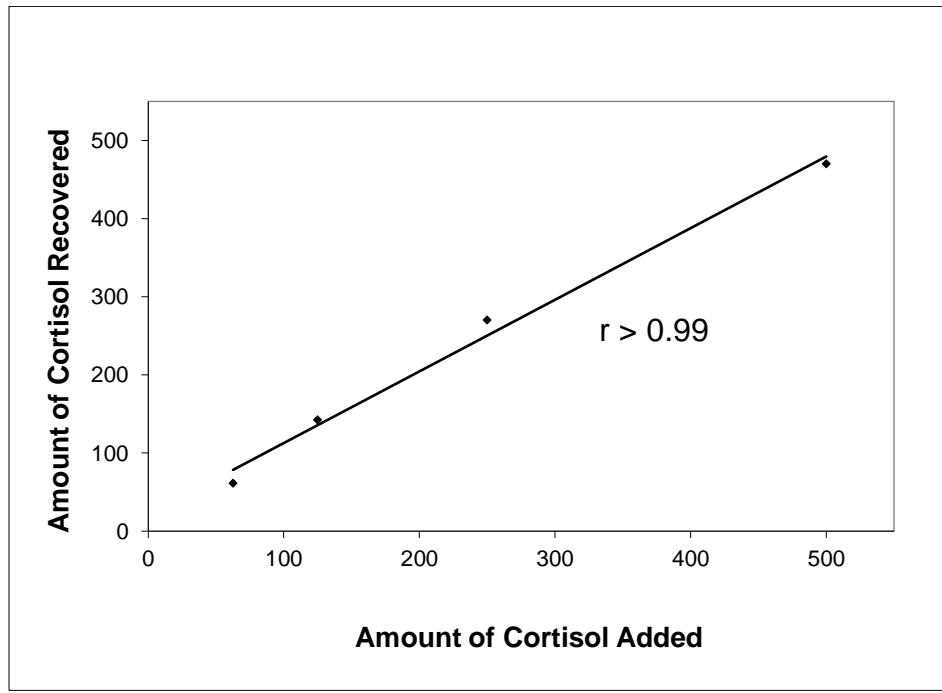
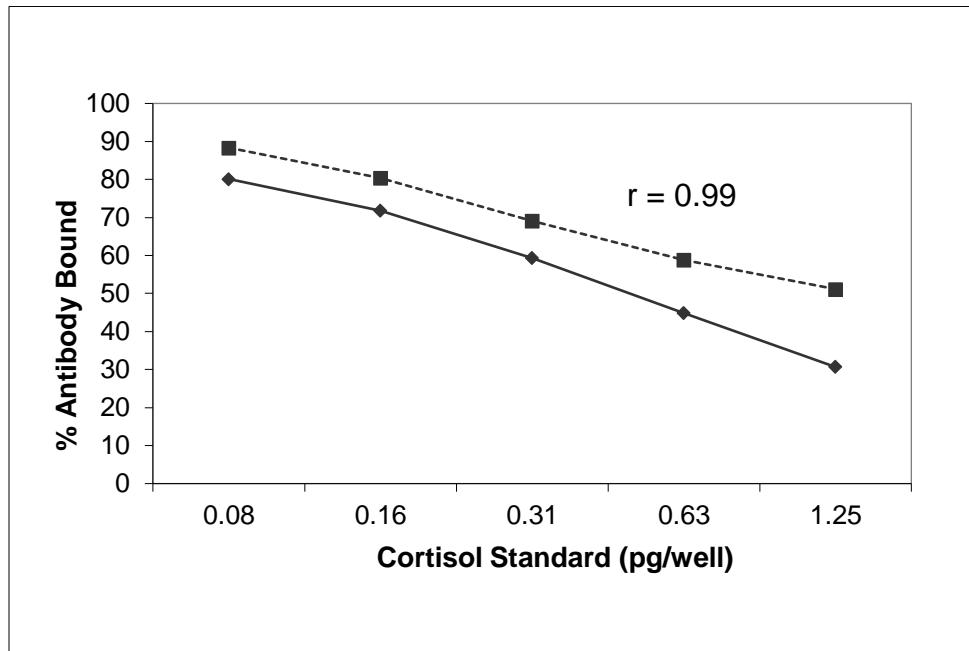


Figure 1.S2 Recovery of exogenous cortisol in pooled a) faecal and b) hair extracts from mountain goats, at Caw Ridge, Alberta (2000-2016).

a)



b)

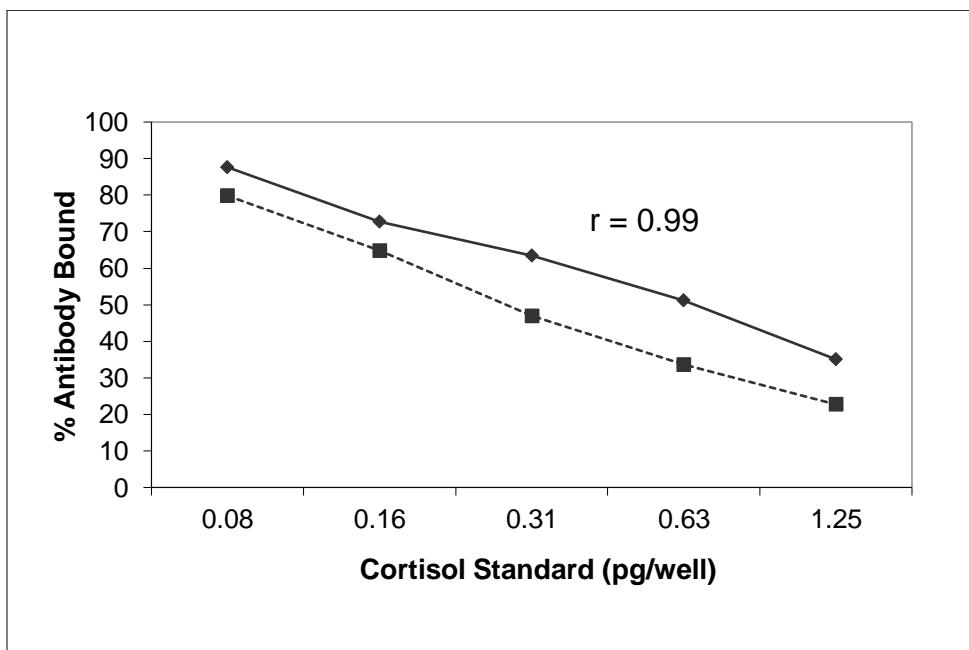


Figure 1.S3 Comparison of serial dilutions of 5 pooled a) faecal and b) hair extracts (squares) from mountain goats, at Caw Ridge, Alberta (2000-2016) to a cortisol standard curve (diamonds) to assess parallelism.

2. Predation risk and mountain goat reproduction: Evidence for stress-induced breeding suppression in a wild ungulate

Frédéric Dulude-de Broin^{* a}, Sandra Hamel^b, Gabriela Mastromonaco^c, and

Steeve D. Côté^a

^a Département de biologie, and Centre d'études Nordiques, Université Laval, Québec, QC
G1V 0A6, Canada

^bDepartment of Arctic and Marine Biology, Faculty of Biosciences, Fisheries, and
Economics, UiT The Arctic University of Norway, 9037 Tromsø, Norway

^c Reproductive Physiology, Toronto Zoo, 361A Old Finch Avenue, Toronto, Ontario,
Canada M1B 5K7

^{*} Corresponding author: frederic.ddb@gmail.com

2.1 Résumé

1. Les effets indirects de la prédation peuvent fortement influencer la reproduction et la démographie d'une espèce proie, mais plusieurs questions persistent quant au mécanisme à l'origine de ces effets en milieu naturel. Spécifiquement, le rôle du stress chronique comme médiateur des effets indirects de la prédation demeure incertain.
2. Bénéficiant de plus de 23 ans de données environnementales, physiologiques et démographiques, nous avons testé l'hypothèse selon laquelle le risque de prédation réduit la reproduction chez la chèvre de montagne en induisant un état de stress chronique. Nous avons effectué des analyses de pistes afin d'évaluer les relations liant le risque de prédation, la concentration en métabolites de glucocorticoïdes dans les fèces ou de cortisol dans les poils et la reproduction en considérant les effets potentiels de la classe d'âge, du sexe, de la masse corporelle, de la saison et des variations individuelles sur la concentration en glucocorticoïdes.
3. Le risque de prédation avait un effet positif direct sur la concentration en métabolites de glucocorticoïdes dans les fèces, laquelle avait un effet négatif direct sur la proportion de femelles reproductives au sein de la population. Le même patron a été observé pour les données utilisant les échantillons de poils, mais ces résultats n'étaient pas concluants probablement en raison de défis méthodologiques associés à l'estimation des concentrations annuelles en cortisol dans les poils.
4. Ces résultats représentent l'un des premiers soutiens empiriques à l'idée que le stress chronique induit par la prédation peut limiter la reproduction chez un ongulé sauvage.

2.2 Abstract

1. Non-consumptive effects of predation can strongly impact reproduction and demography of prey species. Still, the underlying mechanisms that drive non-consumptive effects are not fully understood, and the circumstances under which chronic physiological stress may mediate these effects remain unclear.
2. Benefiting from over 23 years of environmental, physiological and demographic data, we tested the hypothesis that predation risk may impair reproduction of mountain goats through chronic elevation of physiological stress. We conducted path analyses to assess the relationships between predation risk, faecal glucocorticoid metabolites and hair cortisol concentration, and reproduction, while taking into account the potential effects of age class, sex, body mass, season, and within individual variation in glucocorticoid concentration.
3. Predation risk had a direct positive effect on the average annual faecal glucocorticoid concentration in the population, which in turn negatively affected the proportion of reproductive females. The same pattern was observed with hair cortisol concentration, but these results were inconclusive potentially due to methodological challenges in estimating annual average of hair cortisol at the population level.
4. Our study presents one of the first robust evidence that stress-mediated breeding suppression can occur in a wild ungulate following increased predation risk, thereby providing a major insight on the mechanisms underlying non-consumptive effects of predation in wild mammals.

2.3 Introduction

In the presence of predators, prey minimize the risk of being killed by adopting antipredator strategies through changes in behaviour (Lima and Dill, 1990), habitat selection (Fortin et al., 2005; Heithaus and Dill, 2006), diet (Lima and Valone, 1986), morphology (Grant and Bayly, 1981) and physiology (Clinchy et al., 2013; Hawlena and Schmitz, 2010). These adaptations should increase fitness by promoting immediate survival (Lima, 1998), but they often carry physiological costs that can alter body condition (Hik, 1995), reproduction (Sheriff et al., 2009; Zanette et al., 2011) or later survival (McCauley et al., 2011).

Non-consumptive effects of predation can impact demography of prey species as much as direct killing (Preisser et al., 2005), but the underlying mechanism that drive these effects is not yet fully understood (Creel, 2018). Two general hypotheses have been proposed to link predation risk with the demographic costs it induces. The *predation-sensitive foraging hypothesis* states that antipredator behaviours induce a trade-off between food acquisition and safety that reduces available resources for reproduction and survival (Hik, 1995; Sinclair and Arcese, 1995). This hypothesis has been extensively studied and is widely supported in both natural and experimental settings (Brown and Kotler, 2004; Christianson and Creel, 2010; Fortin et al., 2005; Sih, 1980; Zanette et al., 2003). Secondly, the *predation-stress hypothesis* has been proposed, predicting that predator encounters affect reproduction and survival through chronic activation of the stress response (Clinchy et al., 2013).

Stress is a key component of prey response to predation that involves the liberation of glucocorticoid hormones (i.e. mainly cortisol or corticosterone according to species) through stimulation of the hypothalamic–pituitary–adrenal (HPA) axis (Sapolsky et al., 2000). Glucocorticoids are metabolic hormones responsible for the daily and seasonal maintenance of energy balance (Landys et al., 2006), which are also essential for managing energy when dealing with life-threatening situations (Sapolsky et al., 2000). As part of the acute stress response, glucocorticoids promote energy mobilization by stimulating catabolic actions, modulate immune and inflammatory responses, and shutdown long-term functions such as maintenance and reproduction to redirect energy towards emergency functions (Sapolsky et al., 2000; Wingfield et al., 1998). While essential to promote immediate survival, sustained

or chronic activation of the stress response through high predator exposure may severely deter long-term survival and reproduction (MacLeod et al., 2018; Sheriff et al., 2009), which can in turn impact population dynamics of prey species (Boonstra et al., 1998).

The *predation-stress hypothesis* is more recent and has consequently been less studied than the *predation-sensitive foraging hypothesis* (Clinchy et al., 2013). However, it is receiving growing empirical support (Clinchy et al., 2013; Hammerschlag et al., 2017; Yin et al., 2017) and it seems to play a central role in the dramatic demographic fluctuations of the snowshoe hare (*Lepus americanus*) - lynx (*Lynx canadensis*) cycles (Boonstra et al., 1998; MacLeod et al., 2018; Sheriff et al., 2009). Still, in some systems the *predation-stress hypothesis* does not apply. For instance, the decline in elk (*Cervus canadensis*) reproduction following wolf (*Canis lupus*) reintroduction in Yellowstone National Park (Creel et al., 2007) was mainly attributed to the *predation-sensitive foraging hypothesis* (Christianson and Creel, 2010; Fortin et al., 2005; Ripple and Beschta, 2004), with no stress-related evidence (Creel et al., 2009). The ecological conditions under which the *predation-stress hypothesis* is supported are not yet fully understood, but valuable insights may be gained by comparing systems where it is supported with those where it is not (Creel, 2018).

Alpine ungulates are interesting species for studying the *predation-stress hypothesis* because their ability to mitigate variation in predation risk is likely limited. They are unlikely to sustain predator populations alone because they are often distributed in small, discrete populations (Festa-Bianchet et al., 2006). Wide, unpredictable and transient fluctuations of local predation risk are therefore expected because predator abundance may vary independently of prey abundance (Festa-Bianchet et al., 2006; Rominger et al., 2004). Moreover, they are generally confined in alpine “islands” surrounded by unsuitable habitats (Festa-Bianchet et al., 1994). The low availability of alternative habitats could restrain their capacity to reduce predation risk through spatial avoidance (Schmidt and Kuijper, 2015). Laboratory experiments have shown that low predictability and control on exposure to risk increase reactivity to stressful events (Dess et al., 1983; Weiss, 1970), and these factors have recently been suggested as drivers of the *predation-stress hypothesis* (Creel, 2018).

We used physiological, environmental and demographic data from a long-term study of individually marked mountain goats (*Oreamnos americanus*) to investigate non-consumptive effects of predation. We tested the *predation-stress hypothesis* assessing whether predator encounters could impact reproduction through chronic activation of the stress response. Mountain goats are long-lived, iteroparous mammals that adopt a conservative reproductive strategy (Hamel et al., 2010). Their lifetime reproductive success is highly correlated with longevity, and females may skip reproductive opportunities to favour their own body condition and survival (Festa-Bianchet and Côté, 2008). When compared to other ungulates, they appear particularly sensitive to disturbance (Côté, 1996; Côté et al., 1998), and predation, mainly by grizzly bears (*Ursus arctos*), grey wolves and cougars (*Puma concolor*), is thought to be their main cause of mortality (Festa-Bianchet et al., 1994). To avoid being killed, mountain goat strategy is to detect predators at a distance and quickly flee to find refuge in the nearest steep cliff or rocky ledge, which are referred to as escape terrain (Festa-Bianchet and Côté, 2008; Hamel and Côté, 2007). While escape terrains are used as shelters to escape predators, they do not represent alternative habitats because they are confined rocky cliffs that usually do not offer food resources. Because mountain goats adopt a conservative reproductive strategy, are vulnerable to predation, and are likely limited in their ability to predict and control exposure to predators, we hypothesized that high predation risk reduces fertility through the induction of chronic stress. Specifically, we predicted that years with high predation risk are associated with years of high concentration of glucocorticoids, which in turn are associated with a low proportion of reproductive females in the population the following year. Using path analyses to disentangle the direct and indirect effects of predation and 23 years of environmental, physiological and demographic data, our study is unique because it measures both physiological and demographic costs in relation to predation risk, while accounting for resources availability, population size and the effects of age, sex, within individual variation and seasonality on glucocorticoid measurements.

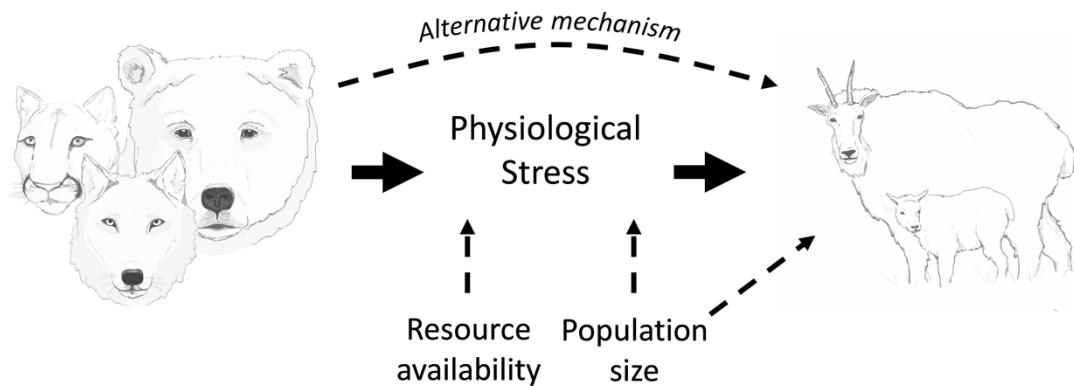


Figure 2.1 The hypothesized causal model linking predation risk to female reproduction in mountain goats. High predation risk is expected to reduce fertility through the induction of chronic physiological stress. Predation risk could also impact reproduction through alternative mechanisms (e.g. trade-off between food and safety). Among environmental covariates, resources availability could impact stress and population size could impact stress and reproduction. Solid arrows represent the predicted relationships based on the predation-stress hypothesis, dashed arrows represent other potential relationships.

2.4 Materials and Methods

2.4.1 Study area and population

We studied mountain goats from 1990 to 2017 at Caw Ridge ($54^{\circ} 03' N$, $119^{\circ} 23' W$), west-central Alberta, Canada. Goats use approximately 28 km^2 of alpine tundra and subalpine open coniferous forests at elevations ranging from 1750 to 2170 m. This population is isolated by large patches of boreal forest with limited goat habitats, in terms of both food resources and escape terrains, and high concentration of predators. Emigration/immigration events are rare and never involved adult females or kids (Festa-Bianchet and Côté, 2008). Summers are short, characterized by a burst of annual vegetation followed by harsh, long-lasting winters. The main predators are grizzly bears, grey wolves and cougars. Other potential predators include black bears (*Ursus americanus*), coyotes (*Canis latrans*), wolverines (*Gulo gulo*) and golden eagles (*Aquila chrysaetos*; Festa-Bianchet and Côté, 2008). Hunting is forbidden in this population since 1969.

2.4.2 Captures and body mass measurements

Goats were captured in traps baited with salt, and marked with individual combinations of ear tags and canvas collars (Côté et al., 1998). Adults were immobilized with xylazine hydrochloride (Haviernick et al., 1998), while goats ≤ 2 years old were physically restrained. From 1996, captures of adult females only occurred to replace damaged collars to avoid kid abandonment (Côté et al., 1998). Most goats were captured once as a yearling and once at 2 years old. Since 1993, over 98% of individuals older than 1 year are marked. The age of nearly all goats included in the study was known because they were first caught as juveniles. Goats first caught as adults were aged by counting horn annuli (Stevens and Houston, 1989). Captured individuals were weighed with a spring scale. Since 2001, goats were also weighed multiple times every year with remotely-controlled electronic platform scales baited with salt, providing >3600 individual masses between 1988 and 2016. To obtain a mass measurement that accounted for seasonal variation and was age-independent, we adjusted individual masses to mid-summer (15 July) using the average seasonal mass gain for each age-class and then performed a polynomial regression of mass on age to use the residuals as a measure of age-specific mass (see Supplementary material 2.8.1).

2.4.3 Reproductive success and demographic data

Weather permitting, daily surveys of the study area were conducted from mid-May to mid-September. Because the resighting probability is at least 98% for this population (Festa-Bianchet et al., 2003), these surveys permitted to precisely determine the survival of every individual through field observations and record population size.

In this population, most females are primiparous at 4 or 5 years old, but some exceptionally reproduce at age 3 (Côté and Festa-Bianchet, 2001a). Because most females give birth between 20 May and 1 June (Côté and Festa-Bianchet, 2001b), we determined the reproductive success of each female though observations of nursing behaviour during intensive surveys from mid-May to mid-June. Prior to parturition, females generally leave the group for 3 to 5 days and isolate themselves in or near escape terrain to give birth to a single offspring (Côté and Festa-Bianchet, 2001b). Successful reproduction was determined

either by direct observation of births or by daily observations of the presence/absence of a kid for each female. While some females might have lost their offspring before we could note its presence, we are confident that this is uncommon because we have very few observations of females that were never seen with a kid but isolated themselves or were lactating at capture (Festa-Bianchet and Côté, 2008). Furthermore, we could not monitor pregnancy right after the rut because it occurs in mid-late November (Mainguy et al., 2008) when the harsh weather conditions of alpine environment make field access very difficult. Therefore, unsuccessful females included females that did not reproduce and potential miscarriage. Because the reproductive status of females that died during winter could not be assessed, these females were not included in the calculation of the proportion of reproductive females.

2.4.4 Predation risk

Every fieldwork day from mid-May to mid-September, the study area was thoroughly scanned using binoculars (10x) and spotting scopes (15-45x) from 13 specific locations along a 9 km transect. These scans were conducted to find goats and predators, which was facilitated by the lack of trees in most of the goat range. To quantify predation risk, we built an index of relative predator presence by dividing the number of predator sightings each year by the number of days in the field (range: 42-108 days). While the main predators of mountain goats are grizzlies, wolves and cougars, we also included sightings of black bears, coyotes and wolverines because they have been seen attacking goats and triggering antipredator behaviours (Festa-Bianchet and Côté 2008). Because lone individuals of these species are likely as threatening to mountain goats as groups of predators, we counted groups such as a bear with cubs or a wolf pack as a single sighting. This avoids the overrepresentation of single stressful events with many individuals relative to multiple stressful events with few individuals. Nevertheless, our index based on the number of sightings was highly correlated with a similar index based on the total count of predators (r [95% CI] = 0.97 [0.93, 0.99], $n=23$ years). For interpretation, we report predator occurrence per 100 days of fieldwork. Predator presence varied from 6 to 58 sightings per 100 days of field work with a median of 19 sightings per 100 days of field work. The total number of sightings across all years were 234 grizzly bears, 48 wolves, 7 cougars, 130 coyotes, 24 black bears and 13 wolverines.

2.4.5 Glucocorticoid measurements

Sample collection

We used 761 faecal samples collected from 2001 to 2016 and 511 samples of guard hair collected from 1994 to 2016. All hair samples and most faecal samples ($n=465$) were collected during capture. Hair with no visible blood, urine or mud contamination were plucked from the rump and/or shoulder and faecal samples were taken directly from the rectum. Although rump and shoulder hair in mountain goats grow during the same period and have similar length and thickness, differences in sample's body location could have increased HCC variability in our study (Heimbürge et al., 2019). On goats captured after the onset of molt, patches of short newly grown hair were avoided. All samples obtained during capture were frozen immediately after handling. Some faecal samples ($n=296$) were also collected opportunistically in the field from 2013 to 2016. Using a 50x camera mounted on a tripod, we recorded the exact location and identity of individuals defecating and retrieved the samples once the goats had left (usually less than 30 minutes after excretion). All opportunistic samples were frozen within 2 to 6 hours. Freezing delay had limited influence on faecal glucocorticoid metabolites (see Supplementary material 2.8.2; Dulude-de Broin et al., 2019). Because of the seasonal pattern in faecal glucocorticoid metabolites concentration (Dulude-de Broin et al., 2019), we adjusted metabolite concentrations to mid-summer (see Supplementary material 2.8.3).

Glucocorticoids analysis

Faecal samples were lyophilised, crushed into powder and homogenized. To extract steroid hormones, we added 5 ml of 80% methanol to 0.20 ± 0.01 g of powdered faecal samples and left them on a rotator plate overnight. After 22 hours, we centrifuged the samples to precipitate faecal material and collected the supernatant that was stored at -20°C until analyses. Prior to quantification by enzyme immunoassay (EIA), extracts were warmed to room temperature and diluted 1:20 in buffer (0.1 mM sodium phosphate buffer, pH 7.0, containing 9 g of NaCl and 1 g of bovine serum albumin per litre).

Only guard hair were included in the analyses (Dulude-de Broin et al., 2019). We cut hair in 5 mm pieces and weighed them in 7 ml scintillation vials. To prevent contamination with

non-visible biological fluids, we washed samples by vortexing them in 100% methanol for 10 s. Immediately thereafter, we extracted steroid hormones by adding 1 ml of 80% methanol per 0.01 g of guard hair, vortexing the vials for 5 s and leaving them on a plate shaker for 24 h (MBI Orbital Shaker; Montreal Biotechnologies Inc., Montreal, Qc, Canada). We then centrifuged the samples and pipetted the supernatants into new vials. Extracts were air dried in fume hood and stored at -20 °C until analysis. Prior to quantification by EIA, samples were warmed to room temperature and reconstituted in assay buffer to obtain a 10-fold increase in concentration. Cortisol is incorporated in hair during anagen, i.e. the period of active growth (Pragst and Balikova, 2006). In mountain goats, growth of the new winter coat starts in June and is completed by November or early December (Déry et al., 2019). The long guard hair collected during summer were therefore used as a measure of previous summer/fall physiological condition.

Faecal glucocorticoid metabolites concentration (FGM) and hair cortisol concentration (HCC) were assayed following the method described in Dulude-de Broin et al. (2019). This method was previously validated in mountain goats for both hair and feces by measuring the adrenal stress response of captive mountain goats following the injection of adrenocorticotrophic hormones (ACTH challenge; Dulude-de Broin et al., 2019). Biochemical validations (parallelism and recovery) of the EIA were done using pooled faecal and hair extracts. The coefficient of variations for control samples within the same plate (intra-assay CV's) were <6% and for control samples across different plates (inter-assay CV's) they were <15% for both hair and faecal samples.

2.4.6 Resource availability

To control for variations in resource availability during summer, we measured the timing of vegetation growth using the date when the peak in faecal crude protein (FCP) occurred. In the highly seasonal alpine environment, date of peak in FCP reflects the timing of spring vegetation green-up (Hamel et al., 2009), which can influence life-history traits of ungulates such as growth or juvenile survival (Pettorelli et al., 2007). Late FCP peak dates indicate delayed vegetation green-up and shorter availability of high-quality resources (Hamel et al., 2009). Each year, we collected faecal samples of 5 to 12 individuals (≥ 1 year old) every 2 to

3 weeks from mid-May to mid-September. Samples were air-dried in paper bags less than 24 hours after excretion. We measured the percentage of protein content in faecal samples using the macro-Kjeldahl acid digestion procedure (AOAC, 1990). For each summer, the relationship between date and the natural logarithm of FCP was assessed using a cubic spline smoother and used to determine the date of maximum FCP (see Hamel et al., 2009).

2.4.7 Statistical analysis

To evaluate the relationships among annual environmental variables, physiology, and reproduction, we needed an annual estimate of glucocorticoids concentration for the population. Age class (≤ 1 year vs adult) and sex had weak to moderate effects on FGM and HCC in this population (Dulude-de Broin et al., 2019). Consequently, simply using the average FGM or HCC among measured individuals could lead to biased results due to variation in age and sex structures (Madliger and Love, 2016). To account for age and sex effects, we estimated average glucocorticoid concentration at the population level using the annual predictions from a linear mixed model. A linear mixed model was fitted separately for FGM and HCC, using the lmer function (R package lme4; Bates et al., 2015) with individual identity (ID) as a random intercept, age class (≤ 1 year vs adult), sex, and their interaction as fixed covariates, and year as a fixed factor (see Supplementary material 2.8.4 for additional information on the models and for explanation on age class selection). We estimated glucocorticoid at the population level using samples from both sexes to keep the largest sample size for obtaining more precise estimates while accounting for the influence of sex. Using a data set limited to samples from females provided similar results (see Supplementary material 2.8.5). To account for the variation in sample sizes among years, the variance associated with each predicted annual estimate was calculated and used as weight in the models where FGM and HCC were fitted as the response variable (see Path analyses below). We excluded the year 2000 because it had only one faecal and one hair sample measured, and variance for this year could not be calculated. The value for that year, however, is plotted in all figures to illustrate its consistency with the results. For HCC analyses, we also excluded year 1999 because no hair samples were collected that year. To evaluate the consistency between average FGM and HCC predicted each year, we performed a Pearson's correlation test for years including both hair and faecal samples (n=15).

Path analyses

To test the hypothesis that predation-induced stress affects reproduction in mountain goats, we conducted path analyses at the population level (Shipley, 2009). A population-level approach is fully relevant in gregarious animals like mountain goats where individuals are generally exposed to very similar environmental conditions, such that predation risk is expected to vary more among years than individuals. Furthermore, our objective was to evaluate whether predation risk could impact the population dynamic of prey species through stress induced breeding suppression, and the detection of an effect at the population level would provide a direct answer to this question. To test our hypothesis at the individual level, we conducted an additional set of path analyses using a reduced data set on adult females only ($n_{faeces}=83$, $n_{hair}=36$) for which both a physiological sample was collected and the following reproductive success was recorded. The individual-level analyses are presented and discussed in Supplementary material 2.8.6.

Based on our knowledge of mountain goat reproduction at Caw Ridge, we built a causal model linking annual environmental variables (predation risk, FCP peak date as a measure of resources availability, and population size), physiological stress evaluated through annual estimates of glucocorticoid concentrations at the population level (either HCC or FGM), and the annual proportion of adult females that successfully reproduced (see Figure 2.1). The causal model did not include a link between FCP peak date and the proportion of reproductive females because there is no evidence for this effect in this population (Hamel et al., 2010). We performed two path analyses: one where physiological stress was based on FGM and one on HCC. We tested the structural relationships among variables based on generalized linear models as described by Shipley (2009) and implemented in the R package “*piecewiseSEM*” (Lefcheck, 2016). For the model where either FGM or HCC was the response variable, we fitted a weighted linear model, with the inverse of the variance as weights. For the model where the proportion of reproductive females was the response, we fitted a generalized linear model with a log link and a quasibinomial distribution to account for overdispersion.

For all models included in the path analyses described above, there was no strong correlation (all r values <0.5) and no multicollinearity (all VIF values <2) among variables included in the same model. FGM data were log-transformed to respect the assumption of normality in model's residuals. To allow comparison of effect size among the tested relationships, we standardized all continuous variables by subtracting the mean and dividing by one standard deviation (Schielzeth, 2010). We evaluated the influence of a variable by examining its estimate and uncertainty based on its 95% compatible interval (95% CI, *sensu* Amrhein et al., 2019), which describes the range of values that are compatible with the data, with values near the estimate being more compatible than values near the limits. We report estimates on the standardized scale, with means and standard deviations allowing back transformation to the unstandardized scale provided in Supplementary material 2.8.7. All statistical analyses were performed in R (R Core Team, 2017).

2.5 Results

Total population size increased from 81 individuals in 1990 to 152 in 2003, remained relatively stable until 2008 and then declined by 80% to reach 34 individuals in 2017 (Figure 2.2A). The proportion of reproductive females fluctuated around 50% until 2002, then progressively declined and was 0 in 2016 (Figure 2.2B). Relative predator occurrence increased in recent years with the seven highest predator sightings per day recorded in the last 11 years of the study (Figure 2.2C). Average age-specific mass of adult females oscillated over the years, with a sharp decrease between 2007 and 2011, but increased afterward when reproduction was at its lowest (Figure 2.2D).

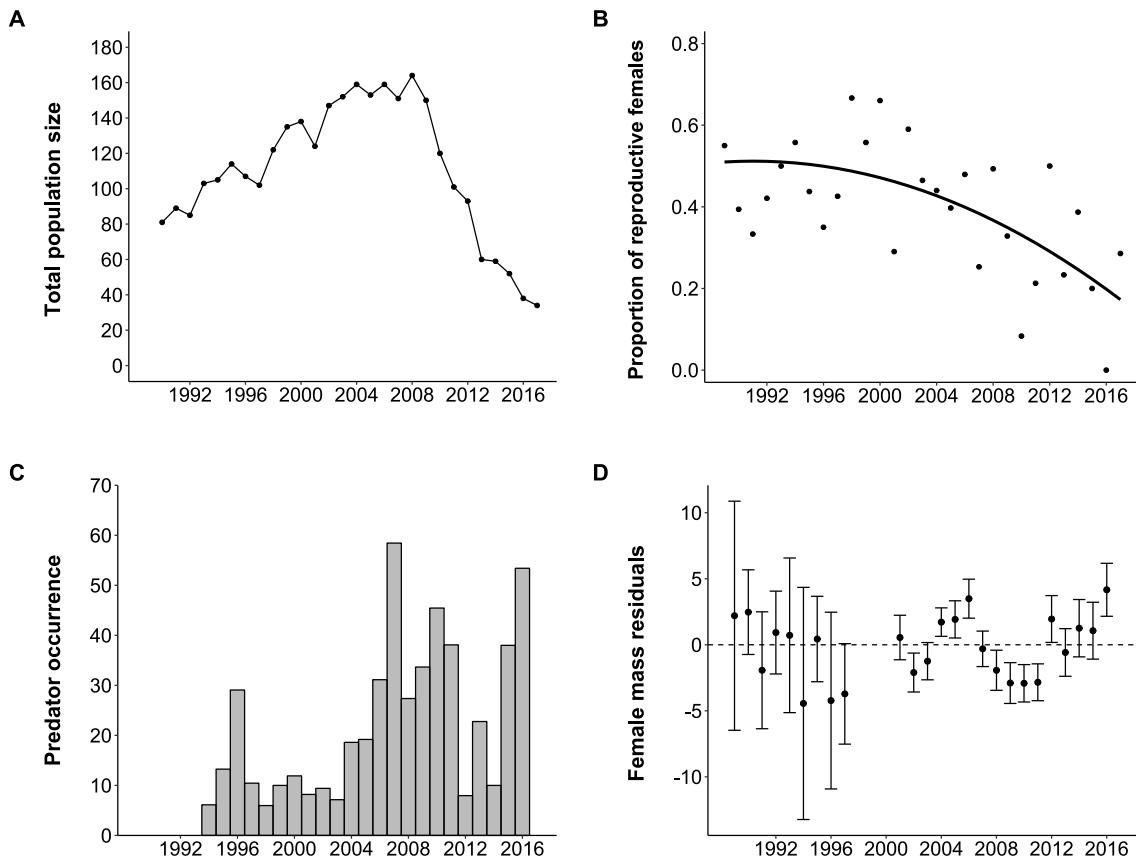


Figure 2.2 Annual variation in demography and predator occurrence at Caw Ridge, Alberta (1989-2017). **A** Total population size on 1 June; **B** Proportion of adult females (≥ 3 years) that gave birth; **C** Number of predator sightings per 100 days of field work (recorded only from 1994); **D** Age-specific mass and 95% CI of adult females (in kg; residuals of mass when accounting for age and seasonal mass variations), where positive values represent years when adult females were heavier than other years during the study period.

Average age-specific mass was more precise after 2001 because we used remotely-controlled electronic platform scales that greatly increased sample size. Annual HCC and FGM were positively correlated (Pearson's r [95% CI] = 0.25 [-0.29, 0.68], $n=15$ years; Figure 2.3). This relationship, however, was uncertain, as the CI indicated compatibility with a weakly negative correlation up to a strongly positive correlation. This is likely due to the large variability in the data, typical of glucocorticoid measurements.

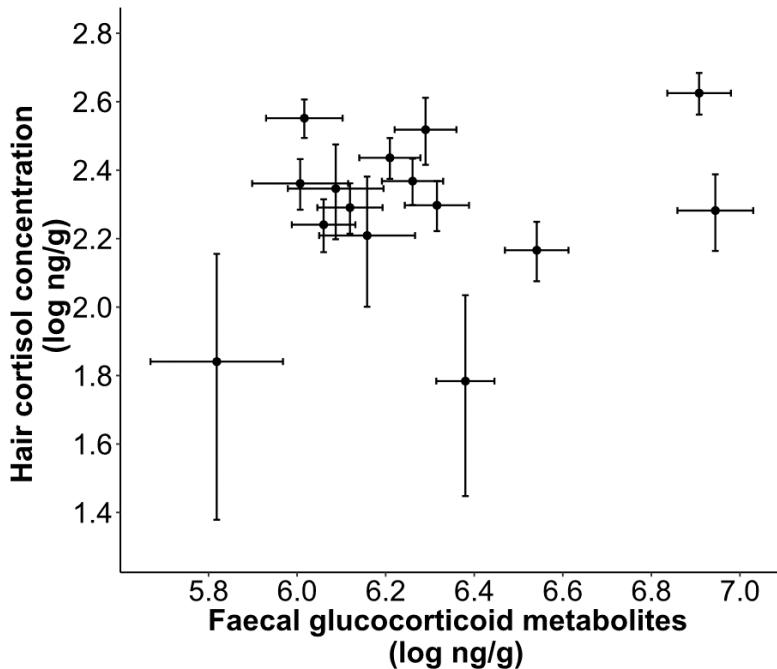
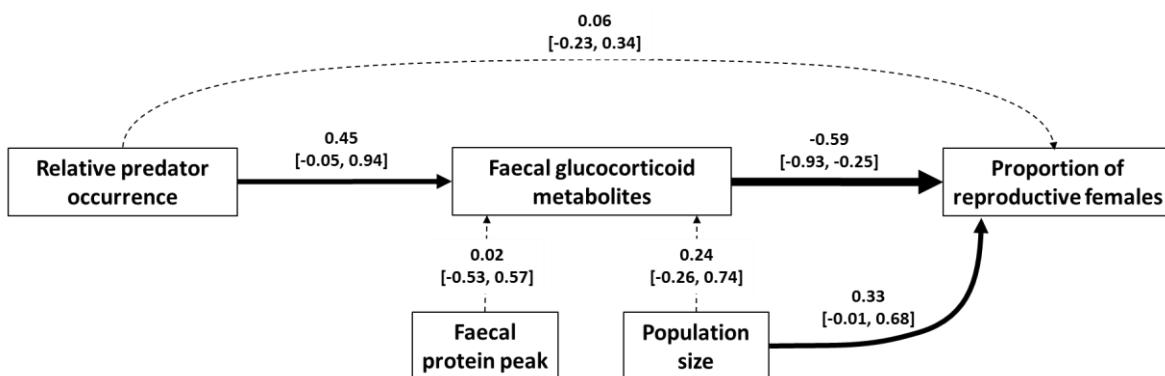


Figure 2.3 Annual faecal glucocorticoid metabolites concentration (FGM) in relation with annual hair cortisol concentration (HCC) in mountain goats, at Caw Ridge, Alberta (2001-2016). The dots represent annual population averages with standard error of FGM and HCC after accounting for age, sex, and individual identity. Log-transformed HCC averages with standard errors are presented to allow same-scale comparison with FGM.

The path analyses revealed that both causal models (Figure 2.4) were consistent with the structure of the data (FGM: Fisher's $C_2=0.151$, $p=0.93$; HCC: Fisher's $C_2=0.402$, $p=0.82$). Relative predator occurrence had a direct positive effect on FGM, which in turn had a direct negative effect on reproduction (Figure 2.4A). There was no direct effect of predator occurrence on reproduction, and among the environmental covariates included, only population size had a direct influence on reproduction (Figure 2.4A). The same directional patterns were observed for HCC, but the relationships were inconclusive (Figure 2.4B).

A



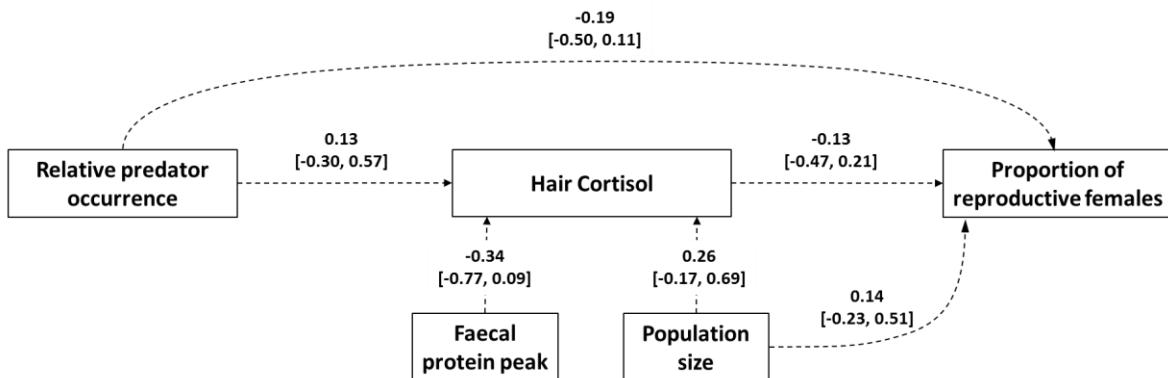
B

Figure 2.4 Direct and indirect relationships between annual environmental variables (predation risk, resource availability measured as faecal crude protein peak, and population size), physiological stress estimated at the population level (**A**: faecal glucocorticoid metabolites concentration, n=761 over 16 years, **B**: hair cortisol concentration, n=511 over 20 years), and annual reproduction of mountain goat females at Caw Ridge, Alberta (1994-2016). Standardized path coefficients [95% CIs] are presented above each path. Thick solid lines represent strong evidence of an effect (95% CIs do not include 0), thin solid lines represent weak evidence (95% CIs marginally include 0), and dotted lines indicate inconclusive relationships (95% CIs widely overlapping 0 in both directions). The models were consistent with the structure of the data (**A**: Fisher's C₂ = 0.151, p-value=0.93, **B**: Fisher's C₂ = 0.308, p-value=0.86).

FGM varied widely among individuals within a year (see the violin plots in Figure 2.5A), with some individuals having low concentrations even at high predation risk. Nonetheless, for 50 additional predator sightings, the average FGM for the population increased by 53% (from 452 ng/g to 694 ng/g; +0.43 on the log scale; Figure 2.5A), which reduced the proportion of reproductive females from over 50% in years with the lowest FGM to less than 20% in years with the highest FGM (Figure 2.5B). Two years, however, had a lower proportion of reproductive females than expected based on their average FGM: the year with no female reproducing, the only time it occurred in this population over 30 years, and the year with the smallest sample size among the years included in the analysis (i.e. year with n=7; Figure 2.5B).

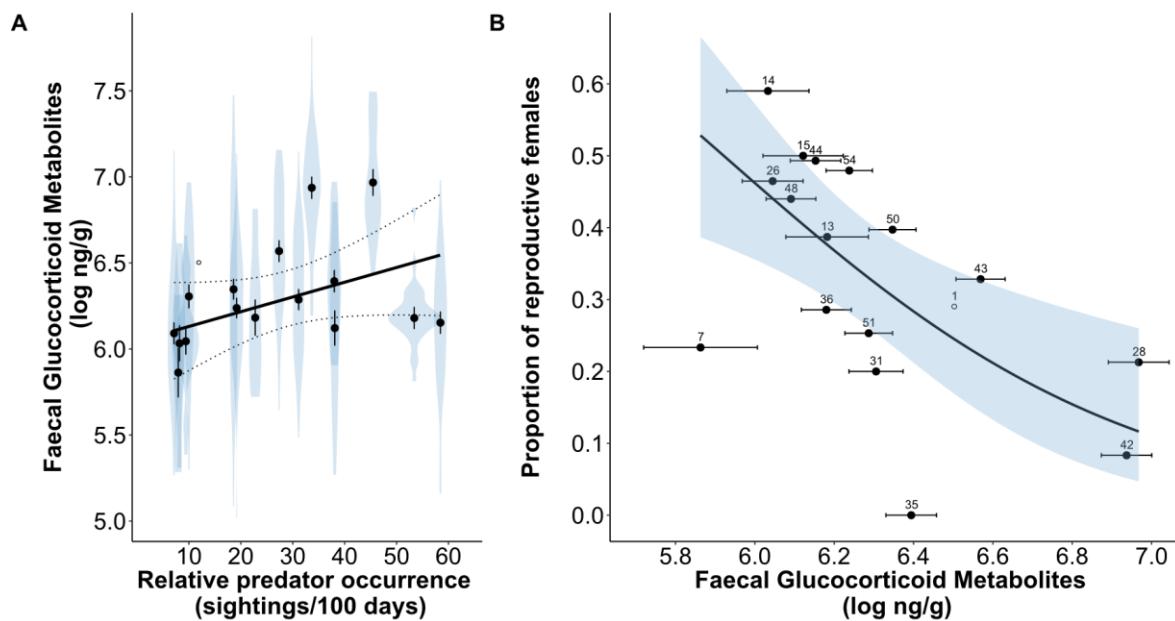


Figure 2.5 Influence of relative predator occurrence on faecal glucocorticoid metabolites concentration (FGM) (**A**), and influence of FGM on the proportion of reproductive females (**B**), in mountain goats, at Caw Ridge, Alberta (1994-2016). The full lines represent the mean model predictions and are presented with their 95% CI (**A**: dotted lines, **B**: polygon). The dots and error bars represent the mean and standard error in annual FGM predicted after accounting for age, sex, and individual identity. The shaded violin (**A**) shows the distribution of the raw data. Numbers are sample sizes, and the hollow dot represents the year with a single sample which was excluded from the analyses, but plotted to show consistency.

2.6 Discussion

Benefiting from 23 years of environmental, physiological and demographic data, our study provides robust evidence that stress-mediated breeding suppression can occur in a wild ungulate following increased predation risk. The specificity of mountain goat ecology coupled with the mechanistic approach and exceptional data set of our study, provide a major insight for understanding non-consumptive effects of predation in wild mammals. Our study also underlines the challenges of working with hormonal data as a substantial part of the variation in FGM and HCC remained unexplained even when accounting for the effects of several variables known to affect glucocorticoid concentrations such as age class, sex, body mass, within-individual variation, seasonal variation, resource availability, and population size.

Predation risk had a direct positive effect on population average FGM which in turn had a

direct negative effect on the proportion of reproductive females. The same pattern was observed with HCC, although these results were inconclusive potentially due to methodological challenges in estimating annual average concentrations (see below). This support for the *predation stress-hypothesis* in mountain goats contrasts with the wolf-elk dynamics observed in Yellowstone National Park where the *predation-sensitive foraging hypothesis* prevailed. Creel (2018) suggested the *control of risk hypothesis* to explain variation in the mechanisms that govern the costs of antipredator responses. According to this theoretical framework, the costs of antipredator responses should be, at least partly, stress mediated when risk cannot be predicted or controlled by proactive responses such as moving to safer areas or shifting periods of activity.

Because the *predation-stress hypothesis* and the *predation-sensitive foraging hypothesis* are not mutually exclusive, our results do not exclude potential additional food mediated effects of predation. Nonetheless, our results are consistent with the *control of risk hypothesis* because mountain goats are likely unable to spatially mitigate variations in predation risk due to the lack of alternative habitats surrounding the “alpine islands” and escape terrain they inhabit. The population studied is confined to 28 km² of suitable habitat surrounded by large and risky patches of boreal forest limiting possibilities for spatial avoidance of threats. Moreover, goats simultaneously face predators that differ in their hunting mode and habitat. For instance, cursorial wolves can attack in open areas, whereas stalking cougars can ambush in forested patches. In this context, spatial mitigation of risk is challenging because avoidance of one predator species may influence vulnerability to a second predator species (Atwood et al., 2009). At a smaller scale, goats might be able to fine-tune their microhabitat use within the landscape by foraging closer to escape terrains (Hamel and Côté, 2007). This should increase the ability to flee once a predator is detected, but does not necessarily reduce the probability of encounter. Consequently, goats still rely on a reactive strategy in which early detection of predators and readiness to escape are key to avoid being killed. Given the limited possibilities for proactive risk mitigation in mountain goats, the evidence of stress mediated costs of antipredator responses in our study are consistent with the *control of risk* framework

One of the preeminent actions of glucocorticoids is to increase the energy readily available to the body, mainly by elevating blood glucose concentrations, increasing blood pressure and cardiac output, and stimulating the catabolic mobilization of lipid and protein stores (Sapolsky et al., 2000). Elevated glucocorticoid concentrations can therefore enhance antipredator response (Wingfield et al., 1998). For example, high glucocorticoid concentrations may be associated with vigilance behaviours (Voellmy et al., 2014), antipredator calling (Blumstein et al., 2006), faster reaction time and increased efficiency of antipredator responses (Thaker et al., 2009). Maintaining high levels of circulating glucocorticoids during periods of elevated predation risk might provide goats with the physiological readiness required to rapidly detect and escape from unexpected predator encounters, thereby potentially increasing individual survival probability (Boonstra, 2012; Wingfield et al., 1998). Nevertheless, the shunt in energy induced by elevated glucocorticoid concentration inevitably entails physiological costs that may impair long-term functions like maintenance, growth or reproduction (Clinchy et al., 2004; Sheriff et al., 2009). We observed a 30% decline in the proportion of reproductive females in years with high compared with low average FGM. Based on long-term monitoring of bighorn sheep (*Ovis canadensis*), Festa-Bianchet et al. (2006) reported marked, transient, and unpredictable periods of increased predation in alpine habitats, suggesting this may be a common threat for small isolated populations of long-lived vertebrates. Our results indicate that during such stochastic periods of elevated risk, non-consumptive effects of predation on reproduction can exacerbate the total impact of predation on prey demography. The potential effects of maternal stress on offspring phenotypes (Love et al., 2012) could also induce long-lasting transgenerational effects of predation, affecting further the population dynamic of prey species (e.g. Sheriff et al., 2010).

Under natural selection, individuals are expected to balance trade-offs among life-history traits to maximize fitness (Stearns, 1992). To evolve, the benefits of elevating glucocorticoids must offset its costs (Boonstra, 2012; Kokko and Ranta, 1996). Mountain goat females produce a single offspring per year, and lifetime reproductive success is strongly correlated with longevity ($r=0.91$, Panagakis et al., 2017). To maximize fitness, females favour their own survival rather than current reproduction (Hamel et al., 2010) and frequently take

reproductive pauses, presumably to compensate for reproductive costs (Hamel et al., 2010; Hamel and Côté, 2009). If elevating glucocorticoids concentration increases survival probability under high predation risk, this mechanism may be an adaptive compromise allowing goats to maximize lifetime reproductive success at the expense of current reproduction.

The effects of chronic stress on reproduction are not limited to females. The elevation in glucocorticoid concentrations we observed could have impacted male fertility by disrupting the gonadal axis or by dampening sexual behaviours (Wingfield and Sapolsky, 2003). In polygynous mating species such as mountain goats, however, only a few fertile males are required to fertilise most females (Mainguy et al., 2009). Fertile females may also mate with multiple males during a single oestrous (Mainguy et al., 2008) thereby increasing the probability of successful fertilisation. Unfortunately, we did not have male fertility data to disentangle the relative influence of chronic stress on male versus female fertility. Therefore, the population-level decline in reproduction we observed could result from the reproductive impairment of either or both sexes.

One major strength of our study is that we accounted for many covariates known to affect glucocorticoid concentrations (Dantzer et al., 2014; Heimbürge et al., 2019; Millspaugh and Washburn, 2004). These covariates are rarely considered in studies on wild populations because they are often unavailable (Dantzer et al., 2014). Still, a substantial part of the variation in glucocorticoids' concentrations remained unexplained. This is expected when using glucocorticoids as a proxy of chronic stress because such measurements integrate both short- and long-term hormonal fluctuations, as well as acute stress responses caused by reactions to transient stressful events (Landys et al., 2006). Using biological matrices that represent an extended period of time such as faeces (~24h) or hair (several months) may help smooth out the variations associated with circadian patterns or other short-term fluctuations (Sheriff et al., 2011a), but they generate additional caveats. For example, variation in metabolic rate or ambient temperature can affect glucocorticoid measurements in faeces (Goymann, 2012; Huber et al., 2003a), while variation in fur growth rate can influence glucocorticoid measurements in hair (Koren et al., 2019). Our results were consistent whether

they were based on faeces or hair samples, but relationships obtained from hair samples were inconclusive. Although the use of hair samples is becoming increasingly popular in animal stress and welfare research (Heimbürge et al., 2019), there are still many unresolved questions regarding the source of confounding variation for this matrix in natural settings (Heimbürge et al., 2019; Koren et al., 2019). We showed previously that hair cortisol concentration can reliably detect five weeks of increased systemic HPA-axis activity in captive mountain goats kept in ideal conditions (Dulude-de Broin et al., 2019). However, hair grown on wild mountain goats were exposed to various weather conditions (e.g. UV-light, rain, mud, snow), which might have increased HCC variability. Furthermore, hair samples were collected from both rump and shoulder, which likely contributed to increase HCC variability. Lastly, the data set used to predict annual HCC ($n=511$ over 20 years) was lower and spread over a larger number of years than that of annual FGM ($n=761$ over 16 years). The small sample size per year could have impacted annual stress estimates obtained with hair samples. Nonetheless, patterns obtained from annual stress estimates based on hair samples were consistent with the clear and conclusive patterns observed with faecal samples. Similarly, the pattern revealed by the individual-level analysis on FGM was inconclusive but consistent with the population-level analyses (Supplementary material 2.8.6). In contrast, the pattern observed with HCC at the individual-level differed from the other path analyses, but this discrepancy was likely attributable to the low sample size and number of years included in the analysis ($n=36$ over 11 years; Supplementary material 2.8.6).

Predation risk effects have been extensively studied in small and weakly mobile animals (Hawlena and Schmitz, 2010; Lima, 1998; Preisser et al., 2005). They have only recently been considered in carnivore-ungulate systems (Moll et al., 2017), likely because of the challenges with experimental manipulation of large mammals (Estes, 1995). As for most studies focusing on prey species but also seeking to monitor wide-ranging predators, our quantification of predation risk was limited. The presence of inconspicuous predators like cougars was likely underestimated compared to canids and bears because of their low detectability. In addition, we could not separate the risk of predators with contrasting hunting mode, which could impact the strength and type of antipredator responses (Schmitz, 2008). Our measure of predation risk also provides an overall estimate of predator presence in the

population during each field season, but it could not account for fine-scale temporal or spatial variation in predation risk. Nonetheless, the positive association between glucocorticoid concentration and predator occurrence suggests the proxy used to quantify predation risk was coarse but suitable, and it allowed identifying predation as a key driver of physiological stress.

Our comprehensive study provides compelling evidence that predation risk negatively impact reproduction in mountain goats through chronic elevation of glucocorticoid concentrations. Indeed, the clear and conclusive pattern revealed by faecal samples at the population level was supported by the inconclusive but consistent pattern observed with hair samples at the population level and faecal samples at the individual level. We propose this mechanism can evolve in long-lived species because their conservative reproductive strategy makes it adaptive to delay reproduction for the benefit of survival when spatial mitigation of risk is limited. Our results suggest prolonged periods of elevated risk, or factors preventing spatial mitigation of risk like habitat fragmentation, could have a substantial deleterious impact on recruitment of prey species and potentially threaten small isolated populations of long-lived ungulates.

2.7 Acknowledgements

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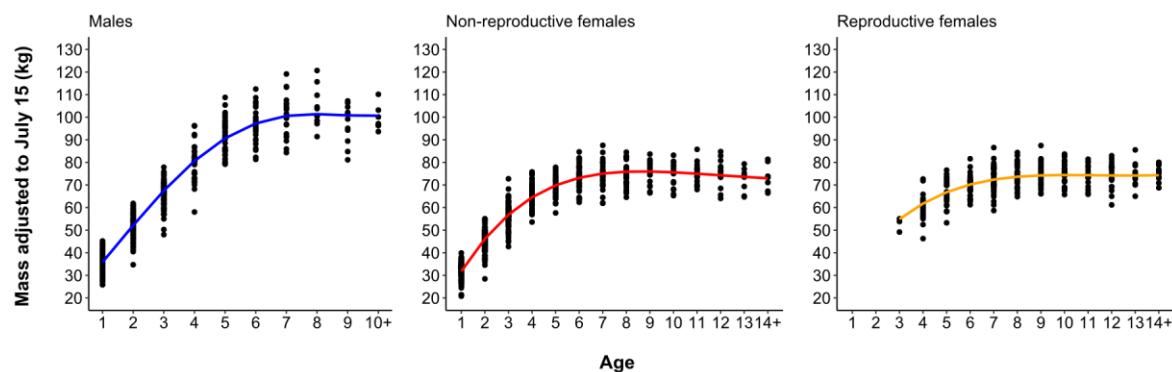
2.8 Supplementary material

2.8.1 Age-specific mass

Mountain goats follow a marked seasonal mass gain during summer and are on average >20 kg heavier in September than in June (Festa-Bianchet and Côté, 2008). Furthermore, mass increases with age until approximately 7 years old in females (Côté and Festa-Bianchet, 2001a; Figure 2.S1) and 6 years old in males (Mainguy and Côté, 2008; Figure 2.S1). Therefore, we adjusted individual masses to mid-summer (15 July) using the average seasonal mass gain for each age-class to compare goats weighed on different dates (Hamel et al., 2009b). Average mass gain was obtained by fitting polynomial regressions with year and individual identity (ID) as random intercepts and day as a fixed effect, using the lmer function of the R package lme4 (Bates et al., 2015). We chose the best polynomial degree by comparing candidate models fitted consecutively from the linear up to the septic function (polynomial of degree 7) using Akaike Information Criterion (AICc), considering the best model as the most parsimonious of equivalent models ($\Delta\text{AICc} < 2$; Burnham and Anderson, 2002). These models were built independently for each combination of age (1 to 6 and ≥ 7 years old for females; 1 to 5 and ≥ 6 years old for males), sex, and reproductive status (for females). When multiple masses were available for an individual in a year, we averaged all adjusted masses for this individual-year. Then, because we needed a mass measurement that was age-independent to have a measure of individual condition that was comparable among individuals, we performed a polynomial regression of mass on age and used the residuals as a measure of age-specific mass. The polynomial regression was fitted with lmer and included year and ID as random intercepts. Masses obtained on males ≥ 10 years and females ≥ 14 years were grouped into one category to keep a minimum of 5 data points for the last age class thereby avoiding the risk of strong influential points at the end of the polynomial relationships. The best polynomial degree was selected with AICc as for the seasonal models. A separate regression was fitted for males, reproductive females, and non-reproductive females (see Figure 2.S1). Therefore, age-specific mass measurements represent individuals

that were heavier or lighter compared with individuals of the same age, sex, and reproductive status, while controlling for seasonal changes in mass.

Figure 2.S1 Mass adjusted to July 15th in relation to age in mountain goats, at Caw Ridge, Alberta (1994-2016). The dots represent the average of all adjusted masses available for each individual-year. The lines are the predictions of the best polynomial regression of mass on age for males, non-reproductive females and reproductive females. Residuals were used as a measure of age-specific mass.



2.8.2 Influence of freezing delay on faecal glucocorticoid metabolites

To evaluate the potential effect of freezing delay on faecal glucocorticoid metabolites, we performed a degradation experiment comparing metabolites concentration of replicates frozen at different times (Dulude-de Broin et al., 2019). The highest difference between replicates frozen immediately and replicates frozen with a delay was -11% [-21%, -0.9%] for replicates exposed for 8 hours to relatively warm (range 7-15°C) and humid conditions (in a backpack in the shade). Faecal metabolites degradation seemed limited by low temperature and was undetectable for replicates exposed to the sun (Dulude-de Broin et al., 2019). We acknowledge a potential variation in glucocorticoid metabolites concentration of 11% for some of the opportunistic samples. Nevertheless, it is unlikely that such a small difference (<0.1 log ng/g for all samples) had a significant impact on our results given the large range of glucocorticoid concentrations measured in our study (from 4.92 to 7.72 log ng/g-dry faeces).

2.8.3 Adjustment for seasonal variation in basal glucocorticoid concentration

Similarly to other vertebrates (reviewed in Romero, 2002), many ungulate species show seasonal variation in basal glucocorticoid concentration (Dalmau and Manteca, 2007; Ingram et al., 1999; Jachowski et al., 2015; Millspaugh et al., 2001; Nilssen et al., 1985). Comparing samples collected on different dates without considering the seasonal rhythm can lead to misinterpretation of FGM results (Jachowski et al., 2015; Millspaugh and Washburn, 2004), but few studies have samples over a long enough duration to account for this confounding effect (Millspaugh and Washburn, 2004). A seasonal pattern of glucocorticoid metabolites concentration was previously detected in our study population, with average faecal metabolites concentration in June and July being 1.5 times higher than in May and 2 times higher than in September (Dulude-de Broin et al., 2019).

Benefiting from 15 years of sample collection, we estimated the effect of the date of sampling on FGM and adjusted measurements to mid-summer (15 July). We fitted a linear mixed model using the lmer function (package lme4 in R; Bates et al., 2015), with year and individual identity as random intercepts, age-class, sex and their interaction included as fixed covariates, and day fitted as a polynomial term. We chose the best polynomial degree by comparing AICc of candidate models fitted consecutively from the linear up to the quintic function (polynomial of degree 5). Day fitted as a quadratic function was the most parsimonious model and was used to adjust faecal glucocorticoids concentration (see Figure 2.S2). Although samples included in this study span from 15 May to 5 September, more than half were collected from mid-June to early July ($n=436$ between 10 June and 7 July), a period during which FGM was relatively stable (see Figure 2.S2). FGM adjusted to 15 July were therefore strongly correlated with unadjusted FGM (estimate [95% CI] = 0.96 [0.95–0.97]; $p < 0.001$; $n=761$). We present results based on adjusted FGM to account for the effect of date on samples collected in May and September, although in this case using unadjusted FGM would produce similar results.

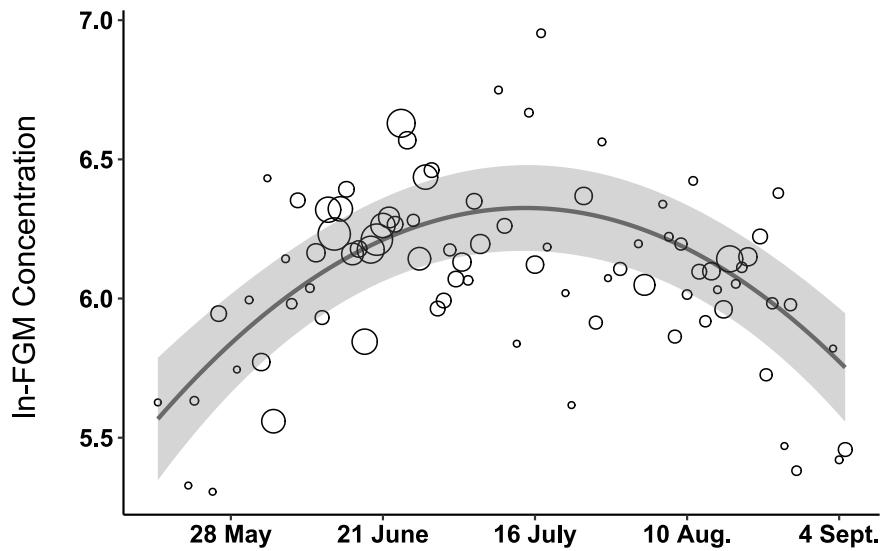


Figure 2.S2 Variation in faecal glucocorticoid metabolites concentration (FGM) during summer in mountain goats, Caw Ridge (Alberta; 2000-2016). Dot size is proportional to sample size, ranging from 1 to 30. The fitted quadratic regression is presented with its 95% CI, including age class and sex as fixed covariates, and goat identity and year as random intercepts. Reproduced with permission from Dulude-de Broin et al., 2019 in *General and Comparative Endocrinology*.

2.8.4 Predictive models for annual FGM and HCC estimates

FGM or HCC were measured only once per individual-year in most years. In 2016, we collected multiple FGM measures per individual as part of another study to assess the effects of age, sex and reproductive status (Dulude-de Broin et al., 2019). Because of this high imbalance in individual repetitions among years, we had to use the average concentration for each individual-year (i.e. one value per individual per year) to reach model convergence. Individual identity as a random intercept accounted for individuals repeated in different years. We modelled age as a two-level category (≤ 1 -year-old vs adult) because the effect of age on FGM and HCC was mostly observed between these categories (Dulude-de Broin et al., 2019). To confirm, we compared models with year as a random intercept, sex as a fixed covariate and age fitted either as two categories (≤ 1 -year-old and older), three categories (≤ 1 -year-old, 2-years-old, and older) or consecutively as a polynomial function from the linear up to the quintic function (polynomial of degree 5) using Akaike Information Criterion (AICc). Age fitted as a two-level category was the most parsimonious model, i.e. the simpler of equivalent models ($\Delta \text{AICc} < 2$), for both FGM and HCC. Furthermore, the predictive model initially included age-specific mass as a covariate to account for potential variation in body condition. Including this variable provided similar predictions for population estimates because it had a negligible effect on FGM and HCC. We therefore did not include age-specific mass for predicting glucocorticoid concentrations at the population level because including this variable substantially reduced sample size as it was not available for all samples (n with/without mass: faeces 697/761, hair 227/511; but see Figure 2.S3 for FGM results based on predictions accounting for age-specific mass).

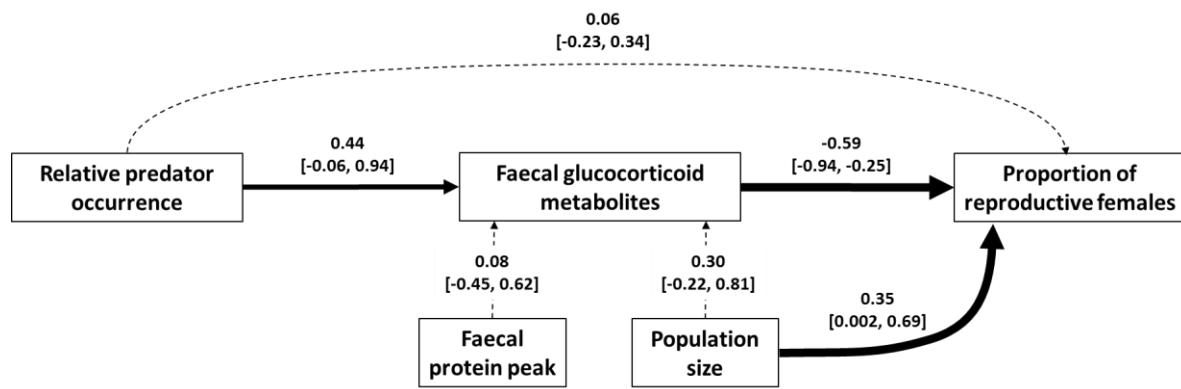


Figure 2.S3 Direct and indirect relationships between annual environmental variables (predation risk, resource availability measured as faecal protein peak, and population size), physiological stress (faecal glucocorticoid metabolites, n=697 over 15 years) estimated at the population level when accounting for variation in individual's age-specific mass, and annual reproduction, in mountain goats at Caw Ridge, Alberta (2001-2016). Standardized path coefficients [95% CIs] are presented above each path. Thick solid lines represent strong evidence of an effect (95% CIs do not include 0), thin solid lines represent weak evidence of an effect (95% CIs marginally include 0), dotted lines indicate inconclusive relationships (95% CIs widely overlapping 0 in both directions). The model was consistent with the structure of the data (Fisher's $C_2 = 0.308$, p-value=0.86).

2.8.5 Path analyses based on a data set limited to samples from females

To obtain annual estimates of glucocorticoids concentration for females only, we used the predictions from a linear mixed model that accounted for the effect of age on a reduced data set limited to samples from females. For FGM, we used the lmer function (R package lme4; Bates et al., 2015) with individual identity (ID) as a random intercept, age class as a fixed covariate, and year as a fixed factor (see Supplementary material 2.8.4). For HCC, individual identity (ID) could not be included as a random intercept because the number of individual repetitions was too small. Therefore, for HCC, the predictions were obtained by fitting a linear model with age class as a fixed covariate and year as a fixed factor. Results using estimates of annual glucocorticoids concentrations based on females only (Figures 2.S4, 2.S5) were similar to results using estimates based on both sexes (Figures 2.4, 2.5).

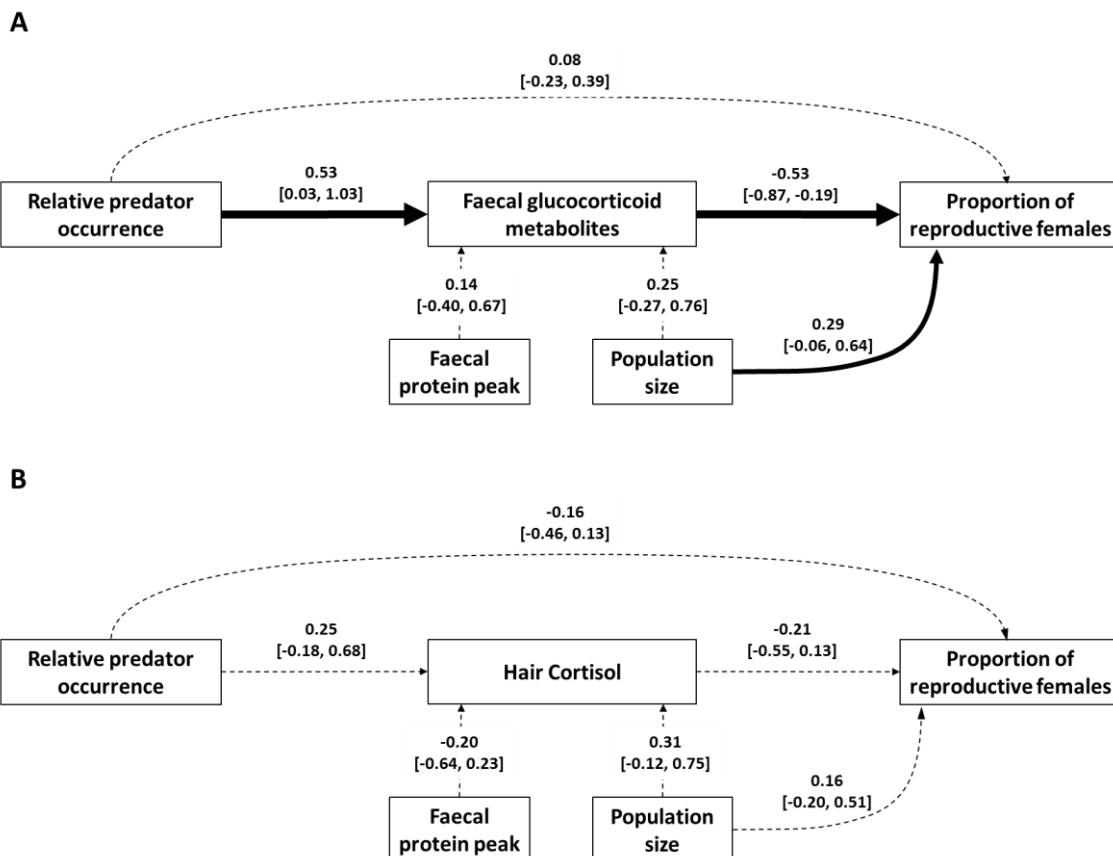


Figure 2.S4 Direct and indirect relationships between annual environmental variables (predation risk, resource availability measured as faecal crude protein peak, and population size), physiological stress estimated at the population level with a data set limited to samples from females (A: faecal glucocorticoid metabolites concentration, B: hair cortisol concentration), and annual reproduction of mountain goat females at Caw Ridge, Alberta (1994-2016). Standardized path coefficients [95% CIs] are presented above each path. Thick solid lines represent strong evidence of an effect (95% CIs do not include 0), thin solid lines represent weak evidence (95% CIs marginally include 0), and dotted lines indicate inconclusive relationships (95% CIs widely overlapping 0 in both directions). The models

were consistent with the structure of the data (**A**: Fisher's $C_2 = 0.157$, p-value=0.93, **B**: Fisher's $C_2 = 0.293$, p-value=0.86).

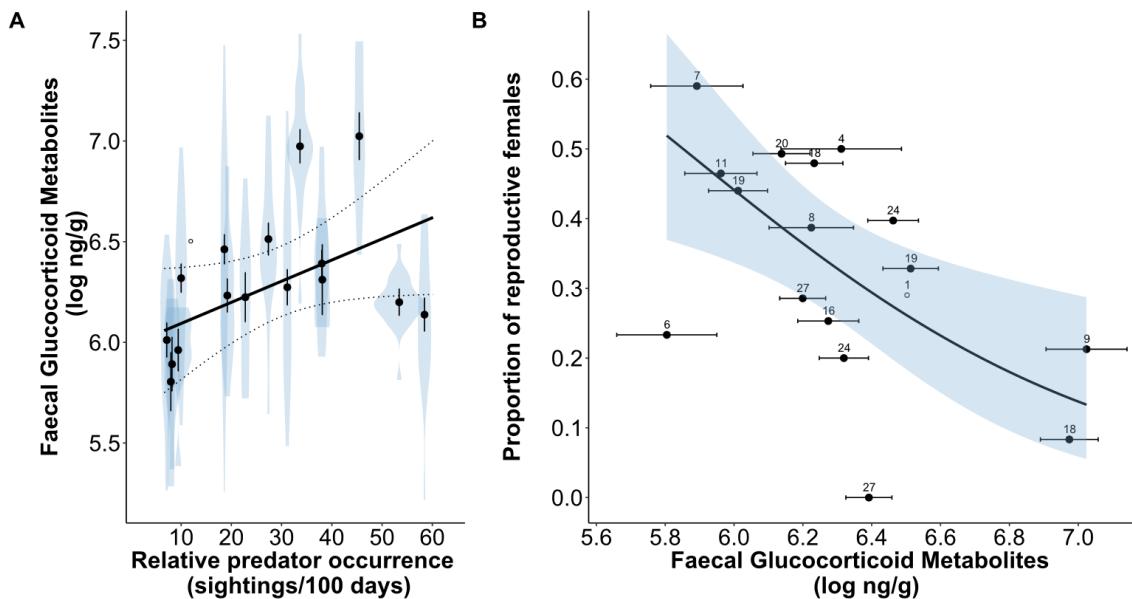


Figure 2.S5 Influence of relative predator occurrence on faecal glucocorticoid metabolites concentration (FGM) estimated with a data set limited to samples from females (**A**), and influence of FGM on the proportion of reproductive females (**B**), in mountain goats, at Caw Ridge, Alberta (2001-2016). The full lines represent the mean model predictions and are presented with their 95% CI (**A**: dotted lines, **B**: polygon). The dots and error bars represent the mean and standard error in annual FGM predicted with female samples after accounting for age, and individual identity. The shaded violin (**A**) shows the distribution of the raw data. Numbers are sample sizes, and the hollow dot represents the year with a single sample.

2.8.6 Path analyses at the individual level

In addition to the path analyses at the population level, we conducted a second set of path analyses using a reduced data set of adult females ($n_{faeces}=83$ over 15 years, $n_{hair}=36$ over 11 years) for which both a physiological sample and the following reproductive success were available at the individual level. These paths were built as generalized multilevel models, including individual identity and year as random intercepts to account for repetitions among individuals and years. For the model where FGM was the response, we fitted a robust linear mixed model with the `rlmer` function in the `robustlmm` R package (Koller, 2016) to account for heavy-tail in the residuals, while the model with HCC was fitted with a linear mixed model with the `lmer` function in `lme4` (Bates et al., 2015). For the model with individual female reproductive success as a response, we fitted a generalized linear model (`glmer` function in `lme4`) with a log link and a binomial distribution. In addition to the relationships specified in the first set of path analyses presented in the main text (hereafter population paths), the second set of path analyses (hereafter individual paths) included as covariates the effect of previous reproductive status (whether a female reproduced the previous year) and age (in 3 categories: young 3–6 years; prime-aged 7–9 years; old ≥ 10 years old) as these variables have been shown to influence female reproductive success in mountain goats (Hamel et al., 2010). Even though female quality, measured with body mass and social rank, has been shown to increase the probability of reproducing in successive years (Hamel et al. 2009), this influence was not as strong as that of previous reproductive status and age (Hamel et al., 2010). Because the low sample size limited the number of parameters that the models could reliably estimate, we did not include mass and social rank as covariates. To evaluate the impact of excluding this potential influence, we added mass as a covariate to the model with the largest sample size (faeces, with $n=83$). The estimate [95% CI] for the influence of mass on reproductive success was 0.35 [-0.58, 1.28], providing confidence that excluding female quality from the individual models based on limited sample size was unlikely to affect the results.

The relationships of the individual path analyses were consistent with the structure of the data (FGM: Fisher's $C_{10}=10.55$, $p=0.39$; HCC: Fisher's $C_8=11.44$, $p=0.18$). Similarly to the results for the population paths, relative predator occurrence had a positive influence on FGM, which in turn had a negative influence on the probability of reproduction (Figure

2.S6A). These relationships, however, were less precise than for the population paths and were inconclusive. For the HCC model at the individual level, we found a direct negative effect of relative predator occurrence on HCC, which in turn seemed to increase a female probability of reproduction, but this last relationship was inconclusive (Figure 2.S6B). The results from the individual path analysis on HCC differs from the other path analyses, but it is based on a very low number of females, as well as fewer years, which means it includes less variation in predator occurrence than the other path analyses.

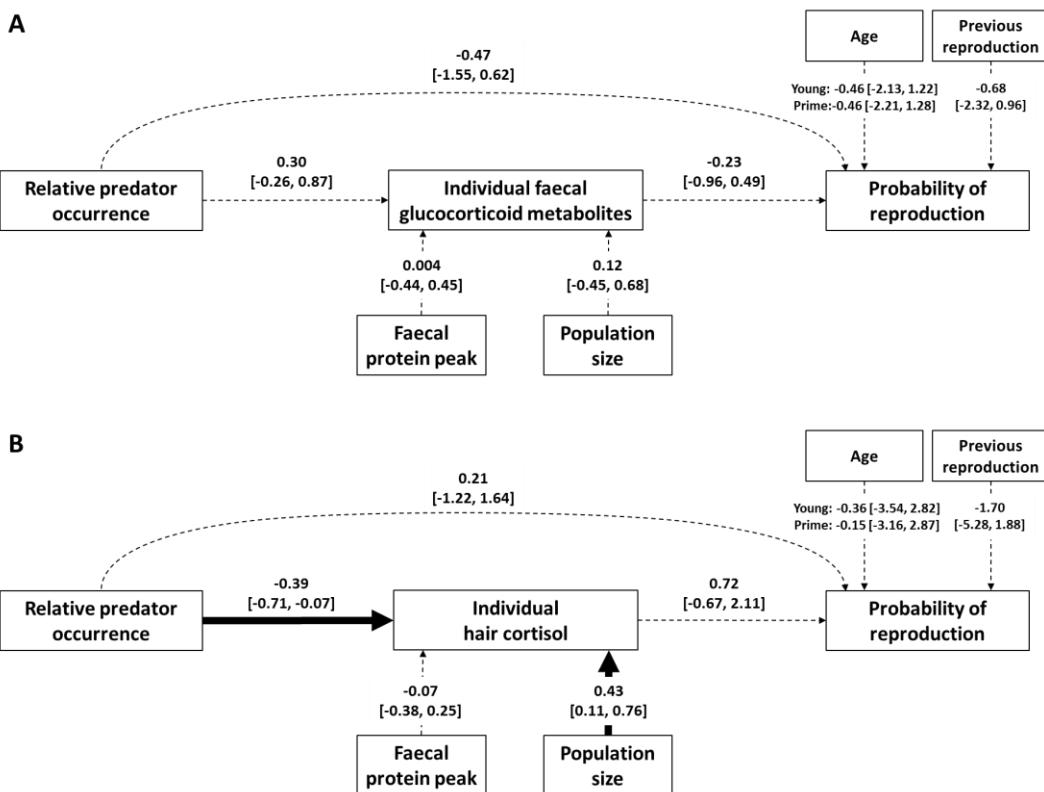


Figure 2.S6 Direct and indirect relationships between annual environmental variables (predation risk, resource availability measured as faecal protein peak, and population size), physiological stress measured at the individual level (A: faecal glucocorticoid metabolites concentration, n=83 over 15 years, B: hair cortisol concentration, n=36 over 11 years), and individual reproductive success, in mountain goats at Caw Ridge, Alberta (1994-2016). Standardized path coefficients [95% CIs] are presented above each path. Thick solid lines represent strong evidence of an effect (95% CIs do not include 0), thin solid lines represent weak evidence of an effect (95% CIs marginally include 0), dotted lines indicate inconclusive relationships (95% CIs widely overlapping 0 in both directions). The models were consistent with the structure of the data (A: Fisher's C₁₀ = 10.55, p-value=0.39, B: Fisher's C₈ = 11.44, p-value=0.18).

2.8.7 Values used for standardizing estimates

Table 2.S1 Means and standard deviations (SD) used to standardized the variables included in a) the population path analyses, b) the individual path analyses.

Variables	Faeces dataset		Hair dataset	
	Mean	SD	Mean	SD
a)				
GC concentration	6.31	0.30	9.06	2.25
Predation	25.93	16.58	21.53	14.90
FCP	42.12	8.81	42.57	10.17
Population size	118.82	43.13	120.57	34.50
b)				
GC concentration	6.35	0.33	9.56	2.89
Predation	31.16	16.59	20.34	14.35
FCP	41.31	6.70	39.19	10.94
Population size	81.35	47.72	138.33	21.31

GC= glucocorticoids; FCP= the date of the peak in faecal crude proteins.

Conclusion

Bénéficiant de 23 ans de données environnementales, physiologiques et démographiques, cette étude représente l'un des premiers supports empiriques robustes à l'idée que la fluctuation du risque de préation en milieu naturel peut agir sur la reproduction d'un ongulé par l'induction d'un état de stress chronique. En plus de contribuer à la compréhension des effets indirects de la préation chez les mammifères, ce mémoire permet de préciser les causes du déclin d'une population sauvage de chèvres de montagne en milieu naturel et de développer de nouveaux outils pour l'étude et la conservation de cet ongulé alpin.

Validation des fèces et des poils comme marqueurs du stress physiologique

Le premier chapitre de ce mémoire visait à valider l'utilisation des fèces et des poils comme marqueurs du stress physiologique chez la chèvre de montagne. Nous avons artificiellement stimulé la sécrétion de glucocorticoïdes par injection d'adrénocorticotrophine (ACTH) et mesuré la réponse hormonale dans les fèces et les poils à l'aide de tests immunologiques spécifiques au cortisol et à la corticostérone. Nous avons également évalué les effets confondants de plusieurs facteurs endogènes et méthodologiques sur les concentrations d'hormones mesurées. Ce chapitre a permis de démontrer que les mesures immunologiques spécifiques au cortisol étaient appropriées pour évaluer le stress physiologique dans les fèces et les poils de la chèvre de montagne, alors que les mesures spécifiques à la corticostérone étaient inadéquates. Parmi les facteurs confondants évalués, l'âge et le sexe avaient une influence sur les concentrations dans les poils, mais plus faible et trop variable pour être détectée au sein des échantillons de fèces. Le type de poil et la date de collecte des fèces entraînaient des variations en concentration de GCs du simple au double et pourraient engendrer un biais méthodologique s'ils n'étaient pas pris en compte. L'expérience de dégradation bactérienne suggère que le délai de congélation est dans une moindre mesure également susceptible d'influencer la mesure des métabolites fécaux de glucocorticoïdes en entraînant des variations maximales de l'ordre de 11%.

Plusieurs études ont souligné l'importance de rigoureusement évaluer la validité d'une méthode de mesures physiologiques pour chaque nouvelle espèce ou matrice biologique

d'intérêt (Johnstone et al., 2012; Kersey et Dehnhard, 2014; Palme, 2005; Sheriff et al., 2011a; Touma et Palme, 2005) et de considérer les effets confondants associés aux caractéristiques individuelles ou aux facteurs méthodologiques (Dantzer et al., 2014; Goymann, 2012; Palme, 2005; Sheriff et al., 2011a). Les résultats du premier chapitre supportent cette idée puisque de multiples variables confondantes ont été identifiées pour chaque matrice biologique et que contrairement aux mesures immunologiques spécifiques au cortisol, celles spécifiques à la corticostérone se sont révélées inadéquates. L'influence de l'âge et du sexe sur les concentrations en glucocorticoïdes suggère que le stress physiologique d'une population devrait être évalué à partir d'échantillons collectés sur des individus d'un même groupe démographique ou en incorporant ces variables individuelles aux analyses. Il en va de même pour le type de poil ou la date de collecte de l'échantillon de fèces. Bien qu'un effet du délai de congélation sur les métabolites fécaux ait été observé, cet effet était faible considérant l'étendue de la variabilité des concentrations en glucocorticoïdes mesurées. Néanmoins, étant donné la possible dégradation des métabolites fécaux, les échantillons devraient être conservés au frais et congelés aussi rapidement que possible. En validant l'utilisation des fèces et des poils comme marqueurs physiologiques des glucocorticoïdes chez la chèvre de montagne et en identifiant les facteurs confondants susceptibles de les influencer, le premier chapitre a permis de poser les bases sur lesquelles sont construites les analyses du deuxième chapitre. Il pourrait également servir d'assise aux futures études s'intéressant à la physiologie de la chèvre de montagne en offrant des outils puissants et pouvant être utilisés de manière non invasive pour évaluer le stress physiologique chez cette espèce.

Influence du stress de prédatation sur la reproduction

Le deuxième chapitre de ce mémoire visait à préciser les causes du déclin de la population de chèvres de montagne de Caw Ridge en se concentrant sur le déclin de la reproduction et à enrichir la compréhension des effets indirects de la prédatation chez les mammifères en milieu naturel. Forts des connaissances acquises lors du premier chapitre, nous avons estimé le stress physiologique annuel de la population en tenant compte de chacune des variables confondantes identifiées lors de la validation. Nous avons alors procédé par analyse de pistes

afin de tester l'hypothèse selon laquelle la prédation influence la reproduction *via* l'induction d'un stress chronique.

Le risque de prédation avait un effet positif direct sur la concentration en glucocorticoïdes estimée annuellement à l'échelle de la population, laquelle avait un effet négatif direct sur la proportion de femelles reproductives. Un patron similaire a été observé pour les échantillons de poils, mais les résultats n'étaient pas concluants, potentiellement en raison de défis méthodologiques affectant les concentrations annuelles estimées à l'échelle de la population. Ces résultats contrastent avec l'absence de stress physiologique qui accompagnait la réduction de la reproduction des wapitis dans le parc de Yellowstone suite à la réintroduction des loups dans l'écosystème (Creel et al., 2009, 2007). En portant attention à ce qui distingue ces deux espèces, il est possible d'identifier les éléments susceptibles de déterminer dans quelles circonstances la prédation agit sur la reproduction *via* le stress (*hypothèse prédation-stress*) et dans quelles circonstances elle agit en induisant un compromis entre l'acquisition de ressources alimentaires et la sécurité (*hypothèse prédation-sélection de ressources*). L'un des aspects majeurs qui distinguent les wapitis de Yellowstone des chèvres de montagne de Caw Ridge est la disponibilité d'habitats alternatifs et la possibilité pour les proies de modifier leur exposition au risque de prédation par des changements dans l'utilisation de l'habitat. Les wapitis de Yellowstone occupent un habitat hautement hétérogène qui leur permet d'utiliser des habitats moins risqués lorsque le risque de prédation augmente (Fortin et al., 2005; Ripple et Beschta, 2004; Schmidt et Kuijper, 2015). À l'opposé, les chèvres de Caw Ridge ne disposent que de 28km² de toundra relativement homogène entourée de forêt boréale hautement risquée, ce qui limite grandement leur capacité à éviter spatialement les variations du risque de prédation. Qui plus est, étant donné la présence de prédateurs qui diffèrent dans leurs stratégies et habitats de chasse, l'évitement d'un type de prédateur pourrait augmenter l'exposition des chèvres à un autre type de prédateur (Atwood et al., 2009). Ainsi, alors que les wapitis semblent pouvoir anticiper et contrôler leur exposition au risque de prédation, les chèvres de montagne semblent contraintes à faire face à la menace et à réagir rapidement pour trouver refuge dans un terrain de fuite lorsqu'un prédateur est détecté. En mobilisant l'énergie vers les fonctions d'urgence de l'organisme, l'état d'alerte associé au stress peut accroître les comportements de vigilance (p.ex. Blumstein et al., 2006; Voellmy et al., 2014) et optimiser la réaction de fuite (p. ex. Thaker et al., 2010, 2009; Uller

et Olsson, 2006) des espèces proies. Les possibilités d'évitement spatial étant restreintes, l'élévation du stress physiologique observé à haut risque de préation pourrait donc augmenter la probabilité de survie des chèvres de montagne. En revanche, la redirection des ressources énergétiques associée à l'état de stress vers les fonctions d'urgence se fait aux dépens de la reproduction annuelle. Un tel mécanisme est cohérent avec la stratégie de reproduction conservative adoptée par la chèvre de montagne chez qui le succès reproducteur à vie dépend principalement de la longévité (Panagakis et al., 2017). Ainsi, les résultats du second chapitre supportent l'idée selon laquelle la capacité d'une proie à prédire et contrôler son exposition au risque est un facteur déterminant favorisant le stress physiologique comme mécanisme anti-préation (Creel, 2018) et suggèrent qu'en raison de leur isolement et du manque d'habitats alternatifs, les ongulés alpins sont particulièrement à risque de subir les effets indirects associés au stress de préation.

Le déclin de la population de Caw Ridge

La proportion de femelles reproductives à Caw Ridge était d'environ 50% lorsque le risque de préation et le niveau de stress de la population étaient faibles et d'environ 20% lorsque le risque de préation et le niveau de stress de la population étaient élevés. Vu l'ampleur de cet effet, il pourrait être tentant de conclure que l'influence de la préation sur la reproduction *via* le stress est à l'origine du déclin de la population. Toutefois, chez les espèces longévives et ne donnant naissance qu'à un seul jeune par année comme la chèvre de montagne, le recrutement annuel ne représente généralement qu'un faible pourcentage de la population totale et les conséquences démographiques d'une réduction de la reproduction peuvent prendre plusieurs années avant d'être visibles (Owen-Smith et Mason, 2005). Un changement de la survie des adultes, généralement très stable d'une année à l'autre (Gaillard et Yoccoz, 2003), est quant à lui susceptible d'engendrer des variations d'abondance beaucoup plus fortes et rapides (Emlen et Pikitch, 1989; Escos et al., 2008; Gaillard et Yoccoz, 2003). Par exemple, les résultats de Hamel et al. (2006), obtenus à partir des prédictions de modèles matriciels de population, montrent que la plupart des populations de chèvres de montagne en Alberta ne peuvent supporter une augmentation de la mortalité des adultes de plus de 1%. Or, la survie adulte de la population de Caw Ridge était environ 5% plus faible de 2004 à 2017 par rapport à la période de 1993 à 2003 (Hamel et Côté, en préparation), suite à

l'augmentation de la présence de prédateurs. Il est donc hautement probable que les effets indirects de la prédation sur la reproduction ne sont pas à eux seuls à l'origine du déclin observé à Caw Ridge, mais qu'ils représentent plutôt l'un des facteurs agissant de concert avec d'autres éléments comme la mortalité directe induite par la prédation.

Limites de l'étude

Au niveau du premier chapitre, la taille d'échantillon de l'expérience de validation était limitée par le nombre d'individus disponibles en captivité. Quatre individus ont été utilisés pour la validation de la méthode avec les poils et trois pour la validation de la méthode avec les fèces. Des tailles d'échantillon similaires sont fréquemment observées lors d'expériences de validation comme la nôtre (Ganswindt et al., 2012, 2003; Ludwig et al., 2013) en raison du défi logistique que représente le maintien en captivité d'espèces de grande taille. Ces tailles d'échantillon sont appropriées pour effectuer la validation physiologique parce que le design robuste de l'expérience restreint l'influence des variables confondantes et que les effets attendus sont larges. Ainsi, la détection de l'injection d'ACTH au sein des fèces et des poils nous a permis de confirmer que ces matrices biologiques reflètent l'activité de l'axe HPA chez la chèvre de montagne et qu'elles peuvent donc être utilisées pour évaluer les concentrations systémiques de glucocorticoïdes. Une fois cet aspect vérifié, c'est avec une taille d'échantillon beaucoup plus grande ($n_{fèces}=761$ sur 16 ans; $n_{poils} = 511$ sur 20 ans) que les effets confondants liés par exemple au sexe ou à l'âge ont été évalués dans le deuxième volet du premier chapitre.

Les rôles des glucocorticoïdes au sein de l'organisme sont multiples et s'étendent au-delà de la réponse d'urgence associée au stress. Ils régissent le rythme circadien (Dallman et al., 1993), varient au gré des besoins saisonniers (Romero, 2002) et peuvent changer avec des facteurs endogènes tels que l'âge, le sexe, la condition physique ou simplement les particularités interindividuelles (Dantzer et al., 2014). L'une des grandes forces de notre étude est l'inclusion de nombreuses covariables connues pour affecter les concentrations en glucocorticoïdes, mais souvent négligées parce que non disponibles (Dantzer et al., 2014). Malgré cela, une portion substantielle de la variabilité au niveau des concentrations en glucocorticoïdes mesurées au sein de notre étude est demeurée inexpliquée par les variables

environnementales considérées. Une grande variabilité résiduelle est attendue lors de l'utilisation des glucocorticoïdes comme indicateurs du stress physiologique parce que la concentration de ces hormones témoigne à la fois de l'état physiologique à court terme et à long terme d'un organisme, en plus de fluctuations passagères causées par des facteurs de stress ponctuels (Landys et al., 2006). L'utilisation de matrices biologiques qui offrent une mesure physiologique intégrée durant plusieurs heures, comme les fèces, ou plusieurs mois, comme les poils, permet d'atténuer la variabilité causée par le rythme circadien et les fluctuations hormonales à court terme (Sheriff et al., 2011a), mais peut entraîner d'autres difficultés. Par exemple, le taux métabolique ou la température ambiante peuvent influencer la mesure des glucocorticoïdes dans les fèces (Goymann, 2012; Huber et al., 2003a), alors que la vitesse de croissance des poils peut influencer leur concentration en glucocorticoïdes (Koren et al., 2019). À l'échelle de la population, les patrons obtenus à partir des échantillons de fèces et à partir des échantillons de poils étaient cohérents, mais seuls ceux obtenus avec les fèces étaient significatifs. Bien que l'utilisation des poils soit de plus en plus commune en écophysiologie (Heimbürge et al., 2019), plusieurs questions persistent quant à la fiabilité de cette méthode en milieu naturel et à l'influence de facteurs confondants (Heimbürge et al., 2019; Koren et al., 2019). Par exemple, le cortisol peut diffuser passivement à l'intérieur des follicules pileux à partir de la sueur, de l'urine ou de la salive (Heimbürge et al., 2019) et les cellules folliculaires ont le potentiel de localement produire du cortisol ne représentant pas nécessairement les concentrations systémiques (Ito et al., 2005). Contrairement aux conditions idéales dans lesquelles étaient maintenues les chèvres lors de la validation expérimentale, les poils récoltés à Caw Ridge étaient exposés à diverses conditions météorologiques (p.ex. rayons-UV, pluie, boue et neige) ce qui pourrait avoir augmenté la variabilité dans les concentrations moyennes de cortisol mesurées. De plus, les échantillons de poils provenaient parfois de la croupe et parfois de l'épaule des chèvres capturées ce qui pourrait également avoir accru la variabilité. Enfin, le jeu de données utilisé pour obtenir les estimés annuels à partir des poils ($n=511$ sur 20 ans) était plus petit et étalé sur un plus grand nombre d'années que celui des fèces ($n=761$ sur 16 ans). La plus petite taille d'échantillon par année pourrait avoir affecté les estimés annuels obtenus à partir des poils. Cependant, bien que les relations obtenues à partir des échantillons de poils n'étaient pas significatives, elles étaient cohérentes avec le patron clair et significatif observé à partir des échantillons de

fèces. De manière similaire, les résultats des analyses individuelles sur les échantillons de fèces n'étaient pas significatifs, mais le patron était cohérent avec les analyses au niveau de la population. En revanche, le patron observé à partir des échantillons de poils au niveau individuel était opposé à celui de toutes les autres analyses, mais cette divergence était probablement imputable à la petite taille d'échantillon et le faible nombre d'années inclus dans cette dernière analyse (n=36 sur 11 ans).

L'influence du stress de prédation sur la reproduction a rarement été étudiée chez les grands mammifères en raison des difficultés associées à la mesure simultanée du risque de prédation, des paramètres physiologiques et des coûts démographiques qui y sont associés (Clinchy et al., 2013; Creel, 2011). Comme pour la plupart des études qui s'intéressent aux espèces proies, tout en faisant le suivi de prédateurs hautement mobiles sur de vastes territoires, notre quantification du risque de prédation était limitée. Par exemple, la présence de prédateurs furtifs comme le cougar a probablement été sous-estimée par rapport à celle des ours, des loups ou des coyotes en raison de leur plus faible probabilité de détection. Nous ne pouvions également pas séparer le risque de prédation d'espèces qui diffèrent dans leur mode de chasse ce qui est susceptible d'affecter le type et la force des réponses anti-prédateurs des proies (Schmitz, 2008). Néanmoins, l'association positive entre la concentration en glucocorticoïdes et le risque de prédation suggère que l'indice utilisé était brut, mais approprié, et il a permis l'identification de la prédation comme un déterminant clé du stress physiologique au sein de notre étude.

Perspectives futures

À la lumière des connaissances acquises au cours de ce mémoire, il importe de discuter des prochaines étapes dans la poursuite de la compréhension des effets indirects de la prédation. Premièrement, l'*hypothèse prédation-stress* et l'*hypothèse prédation-sélection de ressources* ne sont pas mutuellement exclusives. Le soutien des résultats du Chapitre 2 pour l'*hypothèse prédation-stress* n'exclut pas la possibilité que les chèvres adoptent des stratégies comportementales de défense face à la prédation au détriment de l'acquisition de ressources énergétiques. Par exemple, l'augmentation du risque de prédation pourrait forcer les chèvres à être plus vigilantes et à se déplacer plus fréquemment suite à la détection d'un prédateur ce

qui réduirait le temps disponible pour s'alimenter. Il serait donc intéressant de tester l'*hypothèse prédation-sélection de ressources* au sein de la population de Caw Ridge en se concentrant sur les conséquences comportementales du risque de prédation. Pour ce faire, on pourrait évaluer comment le risque de prédation influence l'utilisation de l'espace des chèvres, le temps passé en vigilance, le temps passé en alimentation, ainsi que la qualité et la quantité de ressources alimentaires ingérées. La position géographique et le budget d'activité des groupes pourraient être évalués avec les données d'observations comportementales directes durant l'été issues du suivi à long terme, ce à quoi s'ajouteraient les données spatiales récoltées toute l'année à partir de plusieurs colliers GPS déjà installés. La qualité et la quantité des ressources alimentaires ingérées seraient quant à elles évaluées à partir des échantillons de fèces disponibles (Christianson et Creel, 2010), puis liées à l'utilisation de l'espace et à l'indice annuel de prédation. Ces analyses pourraient être complétées par la récolte d'échantillons 24h après l'utilisation par les chèvres d'habitats plus ou moins risqués ainsi que par des inventaires de végétation au sein de ces habitats afin d'évaluer si les habitats les plus sécuritaires sont aussi les moins favorables au niveau des ressources alimentaires. Bien que certains de ces éléments aient été précédemment étudiés au sein de la population de Caw Ridge (Festa-Bianchet et Côté, 2008; Hamel et Côté, 2007), d'autres sont manquants et ces données n'ont pas été intégrées dans un cadre conceptuel permettant de tester spécifiquement l'hypothèse prédation-sélection de ressources.

Ensuite, l'*hypothèse contrôle du risque* (de l'anglais *control of risk hypothesis*; Creel, 2018) discutée au Chapitre 2 fournit un cadre théorique clair permettant de formuler des hypothèses quant aux mécanismes à l'origine des effets indirects de la prédation. Ainsi, l'*hypothèse prédation-stress* devrait être supportée lorsqu'une proie n'est pas en mesure de prédire ou de contrôler son exposition au risque. Ce mémoire est parmi les premières études à soutenir empiriquement l'*hypothèse prédation-stress* chez un grand mammifère. Il serait profitable d'évaluer à quel point ce mécanisme est répandu en milieu naturel en sélectionnant préférentiellement des espèces qui contrastent dans leur capacité de réponse spatio-temporelle face à la prédation. Par exemple, d'après l'*hypothèse contrôle du risque* et les résultats de cette étude, une élévation subite du risque de prédation serait davantage susceptible d'engendrer un stress chronique chez d'autres espèces d'ongulés alpins comme

le mouflon et le chamois, que chez des espèces hautement mobiles et généralement moins limitées dans la disponibilité d'habitats alternatifs comme le cerf et l'original (*Alces alces*).

Enfin, étant donné les défis logistiques associés à la manipulation expérimentale de grands mammifères, il serait avantageux de coordonner les activités de gestion en milieu naturel avec un suivi physiologique permettant d'en évaluer l'effet. Les mesures de conservation impliquant le contrôle de prédateurs ou encore les variations marquées de pression de chasse constituent des opportunités intéressantes pour tester de manière causale l'influence du stress de prédation sur la démographie des populations de proies adjacentes.

Conclusion générale

Ce mémoire illustre le grand potentiel, mais aussi les défis que représente l'étude de la physiologie en milieu naturel. Un grand potentiel parce qu'elle permet une approche mécanistique des processus écologiques et offre ainsi la possibilité de comprendre les relations qui lient un phénomène à ses conséquences démographiques. Des défis parce que les hormones ont un large éventail d'actions au sein de l'organisme et réagissent conséquemment à de multiples variables qui deviennent confondantes lors de l'étude d'un processus particulier. En utilisant une méthode validée expérimentalement et en tenant compte des facteurs individuels et méthodologiques pouvant affecter la mesure du stress physiologique, ce mémoire aura permis de mieux comprendre les causes du déclin de la reproduction de la population de chèvres de montagne de Caw Ridge et supporte empiriquement l'idée que la fluctuation du risque de prédation en milieu naturel peut agir sur la reproduction par l'induction d'un stress chronique. Nos résultats suggèrent que des périodes prolongées d'élévation du risque de prédation ou l'ajout de contraintes limitant la capacité d'une proie à éviter ses prédateurs pourraient avoir un impact substantiel sur le recrutement des espèces proies et potentiellement menacer la persistance des petites populations d'ongulés en milieu naturel. Nous proposons de poursuivre l'étude des effets indirects de la prédation en évaluant l'*hypothèse prédation-sélection de ressources* au sein de la population de Caw Ridge, en testant l'*hypothèse prédation-stress* sur d'autres espèces qui diffèrent dans leur capacité de réponse spatio-temporelle face à la prédation et en tirant

profit d'éventuelles mesures de gestion exceptionnelles comme le contrôle de prédateurs pour confirmer expérimentalement ces relations.

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