

RESEARCH ARTICLE

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

Frédéric Dulude-de Broin^{1,2}  | Sandra Hamel^{1,3}  | Gabriela F. Mastromonaco⁴ |
Steeve D. Côté^{1,2} 

¹Département de biologie, Université Laval, Québec City, QC, Canada

²Centre d'études nordiques, Québec City, QC, Canada

³Department of Arctic and Marine Biology, Faculty of Biosciences, Fisheries, and Economics, UiT The Arctic University of Norway, Tromsø, Norway

⁴Reproductive Physiology, Toronto Zoo, Toronto, ON, Canada

Correspondence

Frédéric Dulude-de Broin

Email: frederic.ddb@gmail.com

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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.

KEYWORDS

anti-predator behaviour, glucocorticoids, mountain goat, non-consumptive effects, predator-prey, reproductive success, risk effects, stress

1 | INTRODUCTION

In the presence of predators, prey minimize the risk of being killed by adopting anti-predator strategies through changes in behaviour (Lima & Dill, 1990), habitat selection (Fortin et al.,

2005; Heithaus & Dill, 2006), diet (Lima & Valone, 1986), morphology (Grant & Bayly, 1981) and physiology (Clinchy, Sheriff, & Zanette, 2013; Hawlena & 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, Krebs, &

Boonstra, 2009; Zquette, White, Allen, & Clinchy, 2011) or later survival (McCauley, Rowe, & Fortin, 2011).

Non-consumptive effects of predation can impact demography of prey species as much as direct killing (Preisser, Bolnick, & Bernard, 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 anti-predator behaviours induce a trade-off between food acquisition and safety that reduces available resources for reproduction and survival (Hik, 1995; Sinclair & Arcese, 1995). This hypothesis has been extensively studied and is widely supported in both natural and experimental settings (Brown & Kotler, 2004; Christianson & Creel, 2010; Fortin et al., 2005; Sih, 1980; Zquette, Smith, Oort, & Clinchy, 2003). Second, 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, Romero, & Munck, 2000). Glucocorticoids are metabolic hormones responsible for the daily and seasonal maintenance of energy balance (Landys, Ramenofsky, & Wingfield, 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, Krebs, Boonstra, & Sheriff, 2018; Sheriff et al., 2009), which can, in turn, impact population dynamics of prey species (Boonstra, Hik, Singleton, & Tinnikov, 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, Yang, Shang, & Wei, 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, Christianson, Liley, & Winnie, 2007) was mainly attributed to the *predation-sensitive foraging hypothesis* (Christianson & Creel, 2010; Fortin et al., 2005; Ripple & Beschta, 2004), with no stress-related evidence (Creel, Winnie, & Christianson, 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, Coulson, Gaillard, Hogg, & Pelletier, 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, Whitlaw, Weybright, Dunn, & Ballard, 2004). Moreover, they are generally confined in alpine 'islands' surrounded by unsuitable habitats (Festa-Bianchet, Urquhart, & Smith, 1994). The low availability of alternative habitats could restrain their capacity to reduce predation risk through spatial avoidance (Schmidt & Kuijper, 2015). Laboratory experiments have shown that low predictability and control on exposure to risk increase reactivity to stressful events (Dess, Linwick, Patterson, Overmier, & Levine, 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, Côté, & Festa-Bianchet, 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 & Côté, 2008). When compared to other ungulates, they appear particularly sensitive to disturbance (Côté, 1996; Côté, Festa-Bianchet, & Fournier, 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 & Côté, 2008; Hamel & 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.

2 | MATERIALS AND METHODS

2.1 | Study area and population

We studied mountain goats from 1990 to 2017 at Caw Ridge (54°03'N, 119°23'W), west-central Alberta, Canada. Goats use approximately 28 km² of alpine tundra and subalpine open coniferous forests at elevations ranging from 1,750 to 2,170 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 & 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 & Côté, 2008). Hunting is forbidden in this population since 1969.

2.2 | 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, Côté, & Festa-Bianchet, 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 & 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 >3,600 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 Appendix S1).

2.3 | 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, Gaillard, & Côté, 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 & Festa-Bianchet, 2001a). Because most females give birth between 20 May and 1 June (Côté & Festa-Bianchet, 2001b), we determined the reproductive success of each female through observations of nursing behaviour during intensive surveys from mid-May to mid-June. Prior to parturition, females generally leave the group for 3–5 days and isolate themselves in or near escape terrain to give birth to a single offspring (Côté & 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 & Côté, 2008). Furthermore, we could not monitor pregnancy right after the rut because it occurs in mid-late November (Mainguy, Côté, Cardinal, & Houle, 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 | Predation risk

Every fieldwork day from mid-May to mid-September, the study area was thoroughly scanned using binoculars (10×) and spotting scopes (15–45×) 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 anti-predator behaviours (Festa-Bianchet & 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 over-representation 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] = .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.

2.5 | Glucocorticoid measurements

2.5.1 | 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, Kanitz, & Otten, 2019). On goats captured after the onset of moult, 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 50× 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 min after excretion). All opportunistic samples were frozen within 2–6 hr. Freezing delay had limited influence on faecal glucocorticoid metabolites (Appendix S2; Dulude-de Broin, Côté, Whiteside, & Mastro Monaco, 2019). Because of the seasonal pattern in faecal glucocorticoid metabolites concentration (Dulude-de Broin, Côté, et al., 2019), we adjusted metabolite concentrations to mid-summer (see Appendix S3).

2.5.2 | Glucocorticoids analysis

Faecal samples were lyophilized, 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 hr, 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, Côté, 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 hr (MBI Orbital Shaker; Montreal Biotechnologies Inc.). 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, that is, the period of active growth (Pragst & Balikova, 2006). In mountain goats, growth of the new winter coat starts in June and is completed by November or early December (Déry, Hamel, & Côté, 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, Côté, et al. (2019). This method was previously validated in mountain goats for both hair and faeces by measuring the adrenal stress response of captive mountain goats following the injection of adrenocorticotrophic hormones (ACTH challenge; Dulude-de Broin, Côté, 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.

2.6 | 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, Garel, Festa-Bianchet, Gaillard, & Côté, 2009), which can influence life-history traits of ungulates such as growth or juvenile survival (Pettorelli, Pelletier, Hardenberg, Festa-Bianchet, & Côté, 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 5–12 individuals (≥ 1 year old) every 2–3 weeks from mid-May to mid-September. Samples were air-dried in paper bags less than 24 hr 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).

2.7 | 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, Côté, 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 & 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, Mächler, Bolker, & Walker, 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 Appendix S4 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 dataset limited to samples from females provided similar results (see Appendix S5). 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 Section 2.7.1 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 ($n = 15$).

2.7.1 | Path analyses

To test the hypothesis that predation-induced stress affects reproduction in mountain goats, we conducted path analyses at the population

level (Shiple, 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 ($n_{\text{faeces}} = 83$, $n_{\text{hair}} = 36$) for which both a physiological sample was collected and the following reproductive success was recorded. The individual-level analyses are presented and discussed in Appendix S6.

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 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 Shiple (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 quasi-binomial distribution to account for overdispersion.

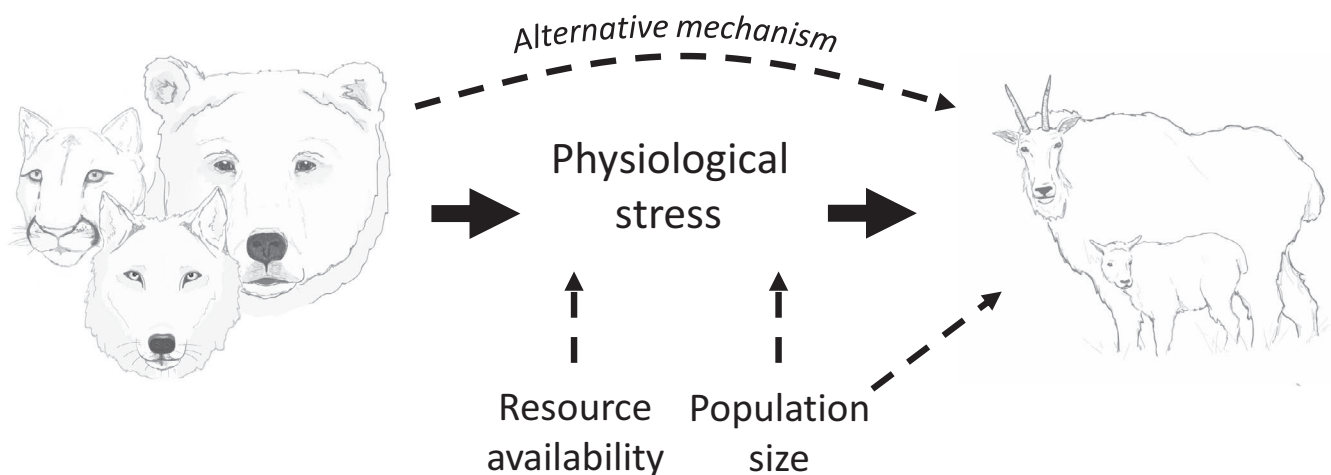


FIGURE 1 The hypothesized causal model linking predation risk to female reproduction in mountain goats. High predation risk is expected to reduce fertility through the induction of chronic physiological stress. Predation risk could also impact reproduction through alternative mechanisms (e.g. trade-off 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

For all models included in the path analyses described above, there was no strong correlation (all $r < .5$) and no multicollinearity (all VIF < 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, Greenland, & McShane, 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 Appendix S7. All statistical analyses were performed in R (R Core Team, 2017).

3 | 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 2a). The proportion of reproductive females fluctuated around 50% until 2002, then progressively declined and was 0 in 2016 (Figure 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 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 2d). 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] = .25 [-0.29, 0.68], $n = 15$ years; Figure 3).

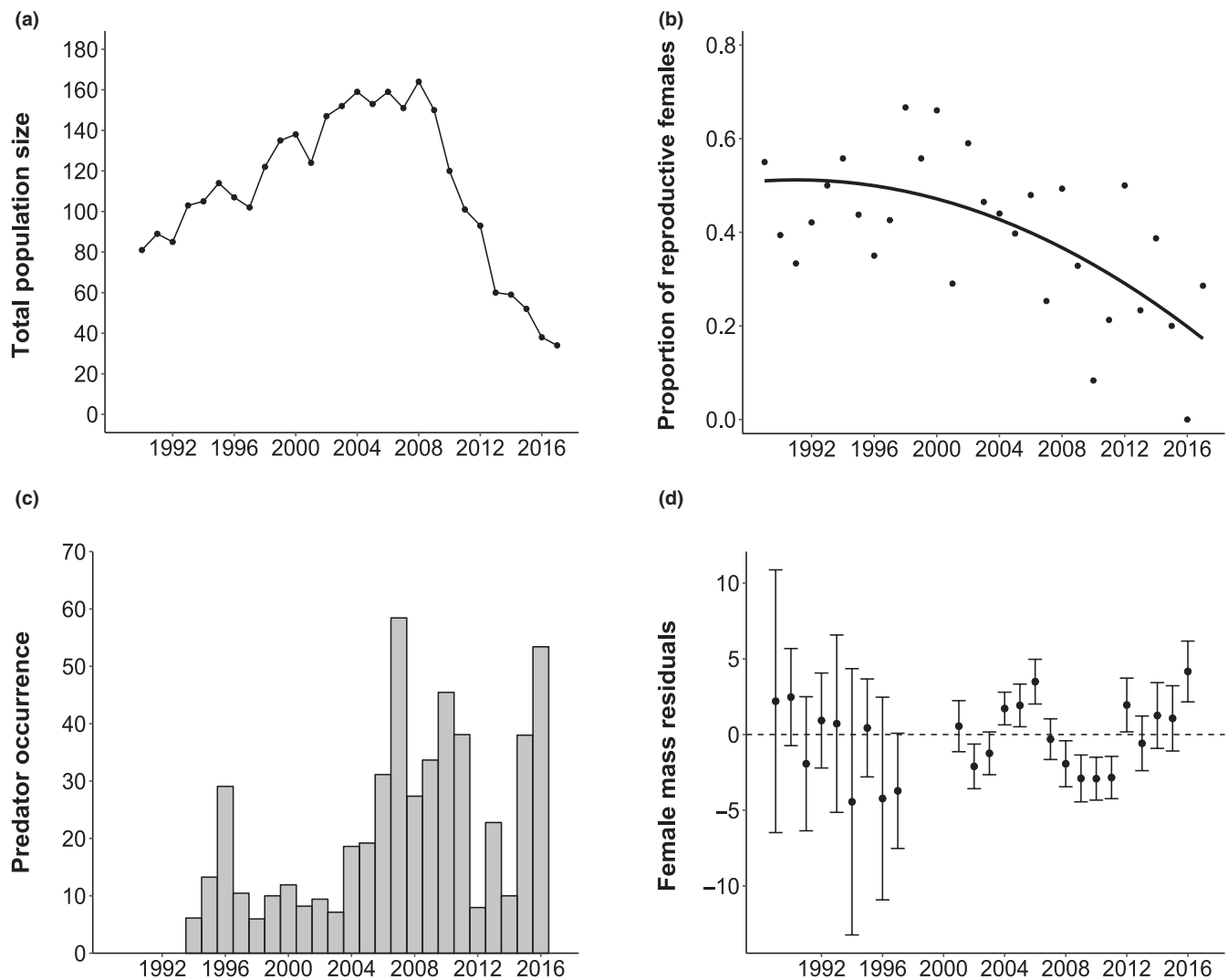


FIGURE 2 Annual variation in demography and predator occurrence at Caw Ridge, Alberta (1989–2017). (a) Total population size on 1 June; (b) Proportion of adult females (≥ 3 years) that gave birth; (c) Number of predator sightings per 100 days of field work (recorded only from 1994); (d) Age-specific 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

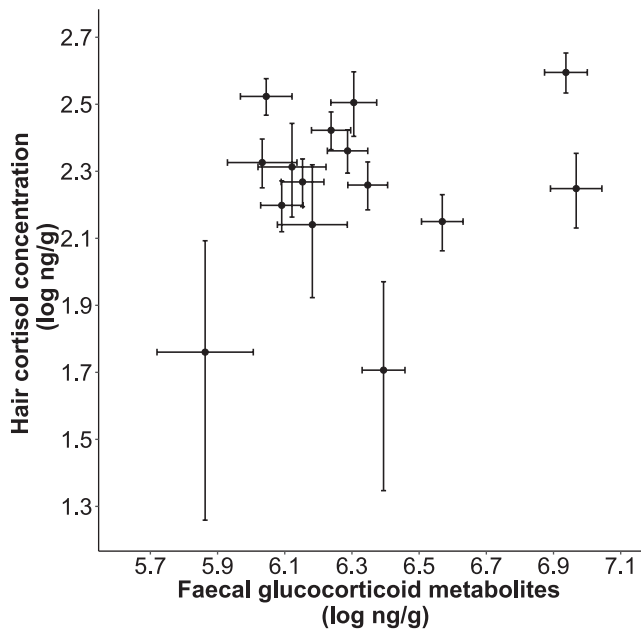


FIGURE 3 Annual faecal glucocorticoid metabolites concentration (FGM) in relation with annual hair cortisol concentration (HCC) in mountain goats, at Caw Ridge, Alberta (2001–2016). The dots represent annual population averages with standard error of FGM and HCC after accounting for age, sex and individual identity. Log-transformed HCC averages with standard errors are presented to allow same-scale comparison with FGM

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.

The path analyses revealed that both causal models (Figure 4) were consistent with the structure of the data (FGM: Fisher's $C_2 = 0.151$, $p = .93$; HCC: Fisher's $C_2 = 0.402$, $p = .82$). Relative predator occurrence had a direct positive effect on FGM, which, in turn, had a direct negative effect on reproduction (Figure 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 4a). The same directional patterns were observed for HCC, but the relationships were inconclusive (Figure 4b).

FGM varied widely among individuals within a year (see the violin plots in Figure 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 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 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 5b).

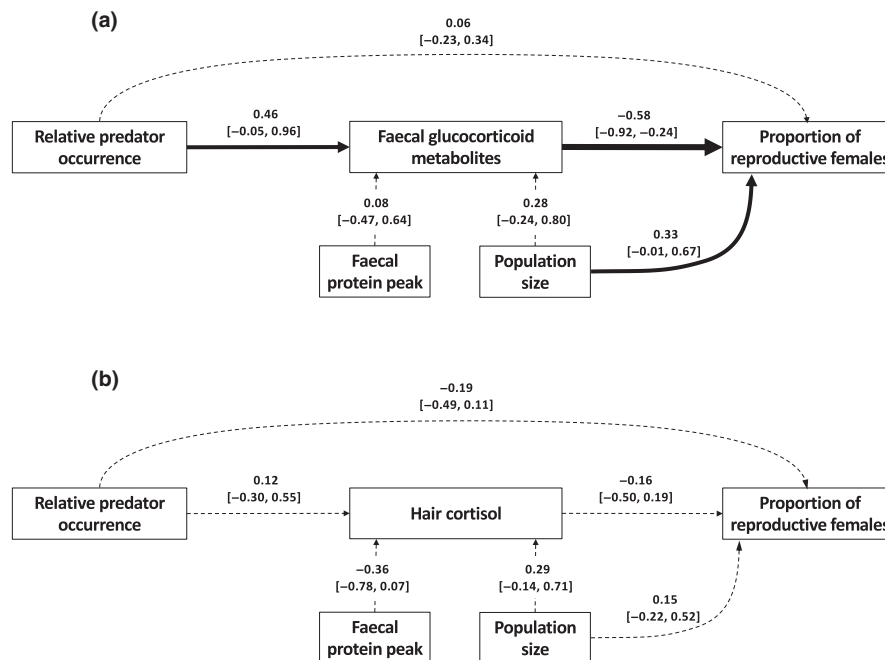


FIGURE 4 Direct and indirect relationships between annual environmental variables (predation risk, resource availability measured as faecal crude protein peak and population size), physiological stress estimated at the population level (a) faecal glucocorticoid metabolites concentration, $n = 761$ over 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 $C_2 = 0.151$, $p = .93$, (b) Fisher's $C_2 = 0.308$, $p = .86$)

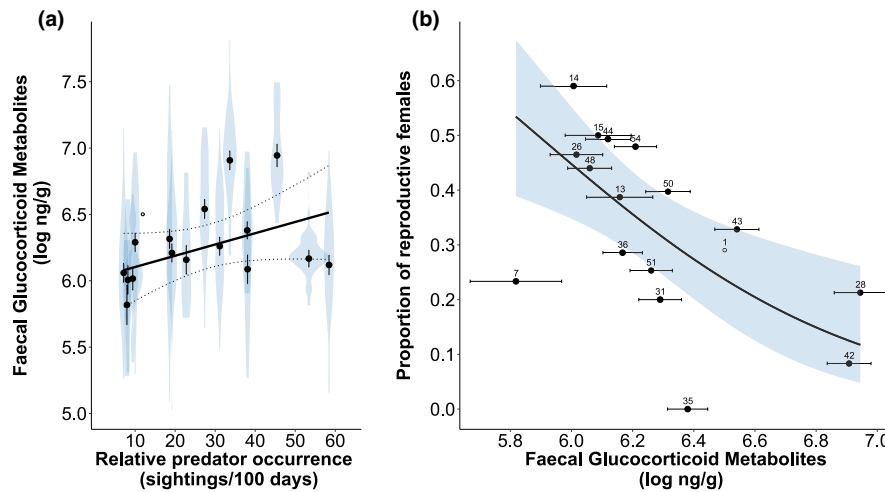


FIGURE 5 Influence of relative predator occurrence on faecal glucocorticoid metabolites concentration (FGM) (a), and influence of FGM on the proportion of reproductive females (b), in mountain goats, at Caw Ridge, Alberta (1994–2016). The full lines represent the mean model predictions and are presented 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

4 | 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 dataset 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 anti-predator responses. According to this theoretical framework, the costs of anti-predator 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, Gese, & Kunkel, 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 & 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 anti-predator 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 anti-predator response (Wingfield et al., 1998). For example, high glucocorticoid concentrations may be associated with vigilance behaviours (Voellmy, Goncalves, Barrette, Monfort, & Manser, 2014), anti-predator calling (Blumstein, Patton, & Saltzman,

2006), faster reaction time and increased efficiency of anti-predator responses (Thaker, Lima, & Hews, 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, 2013; 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, Zanette, Boonstra, Wingfield, & Smith, 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, McGowan, & Sheriff, 2012) could also induce long-lasting transgenerational effects of predation, affecting further the population dynamic of prey species (e.g. Sheriff, Krebs, & Boonstra, 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, 2013; Kokko & Ranta, 1996). Mountain goat females produce a single offspring per year, and lifetime reproductive success is strongly correlated with longevity ($r = .91$, Panagakis, Hamel, & Côté, 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 & Côté, 2009; Hamel et al., 2010). 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 & Sapolsky, 2003). In polygynous mating species such as mountain goats, however, only a few fertile males are required to fertilize most females (Mainguy, Côté, Festa-Bianchet, & Coltman, 2009). Fertile females may also mate with multiple males during a single oestrous (Mainguy et al., 2008), thereby increasing the probability of successful fertilization. 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, Fletcher, Boonstra, & Sheriff, 2014; Heimbürge et al.,

2019; Millsaugh & 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 (~24 hr) or hair (several months) may help smooth out the variations associated with circadian patterns or other short-term fluctuations (Sheriff, Dantzer, Delehanty, Palme, & Boonstra, 2011), but they generate additional caveats. For example, variation in metabolic rate or ambient temperature can affect glucocorticoid measurements in faeces (Goymann, 2012; Huber, Palme, & Arnold, 2003), 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 5 weeks of increased systemic HPA-axis activity in captive mountain goats kept in ideal conditions (Dulude-de Broin, Côté, 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 dataset 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 (Appendix S6). In contrast, the pattern observed with HCC at the individual-level differed from the other path analyses (Appendix S6), but this discrepancy was likely attributable to the low sample size and number of years included in the analysis ($n = 36$ over 11 years; Appendix S6).

Predation risk effects have been extensively studied in small and weakly mobile animals (Hawlana & 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 anti-predator 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.

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AUTHORS' CONTRIBUTIONS

F.D.-d.B. and S.D.C. designed the study. F.D.-d.B., S.D.C. and S.H. collected the data. F.D.-d.B. and G.F.M. realized laboratory analyses. F.D.-d.B. and S.H. performed statistical analyses. F.D.-d.B. wrote the manuscript, and G.F.M., S.H. and S.D.C. commented the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

DATA AVAILABILITY STATEMENT

Data deposited in the Dryad Digital Repository <https://doi.org/10.5061/dryad.3xjsj3txbh> (Dulude-de Broin, Hamel, et al., 2019).

ORCID

Frédéric Dulude-de Broin  <https://orcid.org/0000-0003-4955-156X>

Sandra Hamel  <https://orcid.org/0000-0003-1126-8814>

Steeve D. Côté  <https://orcid.org/0000-0002-4875-1917>

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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