SAHLÉN, ELLINOR SARA. Indirect Effects of Predation in Human-Modified Landscapes. (Under the direction of Christopher DePerno).

Large carnivores have been eradicated from many regions across their historical distribution, a loss that may affect prey species and humans. Here, I explore human-predator-prey interactions, and how large carnivores and humans affect the space use, behavior, and long-term stress of ungulate prey in a Scandinavian region greatly modified by humans. Experimental and observational data are used to quantify behavioral and physiological antipredator responses of prey in areas with and without large carnivores. Further, I synthesize the effects of large carnivores on ecosystems in anthropogenic landscapes, and outline implications of large carnivore recovery for extant prey species and humans.

In southeastern Sweden, where brown bears *Ursus arctos* have continuously been absent since being eradicated over a century ago, I quantified ungulate visitation to artificial feeding sites with different risk treatments (brown bear scent and controls) using camera traps and used sighting distance to investigate the importance of habitat openness for ungulates at the different risk treatments. I determined that prey selected more open habitats in areas where they perceived predation risk to be higher. Increased knowledge about antipredator responses in areas subjected to anthropogenic change is important because these responses may affect prey population dynamics, lower trophic levels, and attitudes toward large carnivores.

In northwest Sweden, I conducted a study to determine brown bear impact on ungulate browsing in one of Scandinavia’s densest brown bear areas. I quantified ungulate browsing in plots with varying levels of risk (assessed by brown bear habitat selection). I distributed plots in which I quantified browsing by counting browsed and unbrowsed twigs in five height classes, and collected data on other habitat related variables (e.g., habitat openness, escape
impediments, and distance to roads). I measured the height of the tallest sapling in the plot, to document potential effects on tree recruitment. Browsing probability was lower in areas that were more intensively used by brown bears, especially where visibility was low. The height of the tallest sapling in plots was shorter in low risk areas, where the probability of browsing was higher. Furthermore, ungulates browsed at lower heights where risk was higher, suggesting that predation risk by brown bears pushed browsing downwards, potentially because of an increased vulnerability when reaching twigs at higher tree heights. The results indicate that predation risk by brown bears may release vegetation from browsing pressure.

Long-term stress is shown to negatively impact growth, immune system, reproduction, and survival, with implications for the management and conservation of wildlife populations. One method to measure long-term stress in mammals is to quantify slowly incorporated stress hormone (cortisol) in their hair, which most likely reflect a long-term average of their stress responses. I sampled moose *Alces alces* across Sweden to study the relative effect of landscape variables and disturbances on moose hair cortisol levels. Hair cortisol levels were higher in the south where the average temperature sum was higher. Also, hair cortisol levels were positively associated with road density; the closer to towns and cities the moose lived, the stronger effect of roads. In contrast, large carnivore occurrence was associated with lower levels of hair cortisol, indicating a lower stress load in these areas, potentially because large carnivore habitat use is higher in areas away from human disturbance.

Human-predator-prey interactions are important to understand to successfully manage systems where these occur, especially in areas where humans and prey species may be inexperienced with large carnivores. Negative attitudes towards large carnivores is one of the
greatest threats against the persistence of large carnivores, and more information about these interactions will be imperative to ameliorate large carnivore impacts upon their return.
Indirect Effects of Predation in Human-Modified Landscapes

by
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To my dad and my son, the past and the future. The wonders of nature were, and will always be, best spent with you.
BIOGRAPHY

Ellinor Sahlén was born on March 8, 1984 in a little town on the Swedish west coast. She was raised in northeastern Sweden, where she developed her love for nature and wildlife. She graduated with a Master’s degree in Management of Fish and Wildlife Populations from the Swedish University of Agricultural Sciences (SLU) in 2010 and worked as a wildlife consultant before starting her doctoral studies at NCSU and SLU. After she completed her doctoral degree Ellinor headed back to northern Sweden to study pedagogics and apply for funding to start her own research project.
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Phantoms of the forest: legacy risk effects of a regionally extinct large carnivore

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Introduction
Although large carnivores are threatened on most continents, these species, along with other large mammals, are currently undergoing a revival in Europe (Ensinger and Vogel 2006; Kindberg et al. 2011). In this process, large carnivores are recolonizing landscapes where they have been absent for centuries and that have been heavily
modified by humans (Chapron et al. 2014). Research on predator–prey interactions has been initiated in North American (Berger 1978; Altendorf et al. 2001; Cred et al. 2005; Reschla and Ripple 2008; Halofsky and Ripple 2008; Lasley et al. 2014) and African ecosystems (Underwood 1982; Valeix et al. 2009; Thaker et al. 2011); however, information about the consequences of carnivores on the behavior and space use of their ungulate prey in heavily human-modified European landscapes is lacking. Here, we recognize two aspects that we consider particularly relevant; prey naïveté toward recolonizing carnivores, and human alterations to the perceived "landscape of fear" (Laundré et al. 2001).

To avoid predation, prey may adjust behavior, morphology, or physiology (Lima 1998; Kunkel and Pletscher 2000; Relyea 2001; Jayakody et al. 2008; Abate et al. 2010; Hossie et al. 2010). Such responses may carry indirect costs by reducing long-term survival, growth, and reproduction (Boonstra et al. 1998; Laundré et al. 2001; Creel et al. 2007) with population effects potentially exceeding those of direct predation (Creel and Christianson 2008). It has been suggested that prey species may lose their antipredator behavior over time if predators disappear from the system (Sih et al. 2010). Therefore, the losses of carnivores in Europe during the last centuries may have resulted in naïve prey that fails to properly respond to predation risk (Berger et al. 2001; Sand et al. 2006). If antipredator behaviors are lost, prey may be more susceptible to predation if predators return (Berger et al. 2001), which may affect interspecific interactions and population dynamics, if only for a transient period. The alternative hypothesis is that prey maintain the ability to recognize their extinct predators for a long time (Li et al. 2011; Chamalîl-Jamnes et al. 2014), perhaps due to an innate fear of predators (Ferro et al. 2011).

In addition to the possibility of prey naïveté, anthropogenic landscape alterations may strongly influence prey responses to predation risk. Landscape features mediate risk (Poyse 1994; Kunkel and Pletscher 2000; Jayakody et al. 2008) and prey may perceive an altered "landscape of fear" (Laundré et al. 2001). In the presence of predators, the predation risk perceived by prey varies depending upon features such as terrain, barriers, and habitat types (Laundré et al. 2001). Habitat openness plays a particularly important role in mediating predator–prey interactions, by affecting vigilance levels (Jayakody et al. 2008) and prey distribution (Valeix et al. 2009; Laundré et al. 2010). For example, sites with less horizontal cover have been shown to be perceived as risky areas by elk Cervus canadensis and moose Alces alces in terms of wolf predation (Kunkel and Pletscher 2000; Cred et al. 2005). However, other studies have failed to detect an effect of habitat openness on antipredator response in closed forest systems with little human impact (Kuijper et al. 2014). Instead they concluded that escape impediments increased perceived predation risk (Kuijper et al. 2013). Anthropogenic landscape change in Europe is extensive, and forestry or agricultural practices have increased the variation of habitat openness in forested landscapes through the construction of forest clear-cuts and agricultural fields (Estreguil et al. 2013). Such alterations may increase opportunities for ungulates to use the variation in habitat openness to reduce predation risk. Currently, information on antipredator responses and risk effects in human-modified European landscapes is largely lacking (but see Lone et al. 2014).

Our objective was to explore the antipredator behavior of prey species in a diverse community of European ungulates and assess how habitat openness altered the nature of their responses. To test this, we experimentally introduced the scent of brown bear (a historically native but now locally extinct predator), and a novel nonpredatory scent, in a highly human-modified landscape in southeastern Sweden and evaluated the responses of five sympatric ungulate species.

Material and Methods

Study area

Because of the absence of large carnivores and high diversity of sympatric ungulate species we conducted our study in Södermanland County in southeastern Sweden (Fig. 1). The landscape is forest dominated but highly fragmented with agricultural lands and clearcuts, which form a heterogeneous patch work of closed forest and open land (see inset in Fig. 1). The forests are mainly composed of boreal coniferous production stands of Scots pine Pinus sylvestris and Norway spruce Picea abies; however, numerous broad-leaved tree species occur throughout the study area, including birch Betula spp., alder Alnus spp., oak Quercus robur, rowan Sorbus aucuparia, and aspen Populus tremula. In Sweden, populations of brown bear and wolf Canis lupus are recovering and recolonizing their historic ranges. Both species were eradicated from the study area over 170 years ago (Statistics Sweden 1984; Swenson et al. 1995) and have not returned. The outer range of the nearest established brown bear population was 100–150 km away from the study area and the nearest wolf pack >50 km (SEPA 2015). Bear sightings in the vicinity of the study area are rare (<10 years ago). The occurrence of wolves wandering through the study area is likely slightly higher. Most ungulates in the study area are unlikely to have ever encountered a bear or a wolf. The sympatric ungulate species in the study area include roe deer Capreolus capreolus, red deer Cervus elaphus, fallow deer
Dama dama, moose A. leu alces, and wild boar Sus scrofa. All species are native to Sweden, except fallow deer, which was introduced in Sweden in the 1570s as a game species from its original Holocene distribution in the Mediterranean and Persia (Dolman and Waber 2008). Relative abundance estimates indicate that fallow deer constitute 61% of the ungulate community, wild boar 23%, roe deer 8%, red deer 5%, and moose 3% (Öster Malma 2013). Red fox Vulpes vulpes and lynx Lynx lynx occur in the area (although the latter is rare). Both species can predate on ungulates, especially lawns. Predator scent as such is thus not novel to the ungulates in the area.

We conducted the study during spring 2013 (March 6–April 19). The coldest temperatures occurred in March (min: \(-18.4^\circ\text{C}\), max: \(8.4^\circ\text{C}\)) and became successively warmer at the onset of spring during April (min: \(-9.4^\circ\text{C}\), max: \(14.1^\circ\text{C}\)). However, temperatures remained relatively low throughout most of our study period, resulting in a packed, icy snow crust covering the ground. For more than 5 years, extensive supplemental feeding of ungulates have occurred throughout the study area during winter and early spring, with ungulates readily using these sites and accustomed to human disturbance and scent. Landowners distributed silage to feeding sites in the area throughout the course of our study period (due to the persistently cold weather).

**Study design**

We used camera traps to document ungulate visitation to 30 artificial feeding sites in forested habitats. The same type of feed (wheat silage) was used at all sites, and feed piles (bales) were distributed by local landowners across an area of roughly 25 m² at each site. All feeding sites had access roads that were used by humans in the area as thoroughfare, for recreation, and distribution of feed. The study was conducted outside of hunting season, and feeding sites were never close to a settlement (>1 km away).
The influence of direct human activity on our results would have been minimal. Each site was exposed to three weekly scent treatments conducted in a block design including brown bear scent, reindeer scent, and controls. We used the reindeer scent treatment as a noncarnivore novel scent; reindeer are confined to northern Sweden and do not occur in the study area (>250 km away). Hence, each site hosted all three treatments during three consecutive weeks (1 week per treatment). We chose this design so that we could easily correct for site effects. Logistics did not allow us to monitor all 30 sites at the same time. Therefore, we conducted the experiment in two rounds: 15 sites were monitored for 3 weeks during March 9–March 27 and 15 sites for 3 weeks during March 28–April 19. During each week, equal amount of sites were exposed to the three different treatments: five exposed to brown bear scent, five to reindeer scent, and five without scent. Additionally, we arranged the treatments so the nearest sites would have different treatments during the same week.

Apellén et al. (2005) suggested that fur has longer lasting effects on prey behavior than feces or urine. Brown bears deposit scent from sebaceous and apocrine glands by rubbing trees, which is used for chemical communications with conspecifics (Clapham et al. 2013), and may function as scent cues for prey species. We mimicked scent-marking by brown bear by attaching pieces of bear pelt to trees. We used pieces of fresh pelt from wild brown bears (provided by the National Veterinary Institute, Sweden) and reindeer (provided by an anonymous Sami reindeer herder) to introduce carnivore and noncarnivore novel scents into our study area. The pelts were cut into pieces (15 x 15 cm) and nailed to small 15 x 15 cm removable wooden plates, with a 10 x 15 cm "roof" that reduced the effects of snow, rain, and ice. To retain their scent, the pieces of pelt were kept in a freezer (−20°C) until used in the field. Also, low temperatures counteracted decay of pelt pieces. The scent structures were attached at breast height to tree trunks using metal wire. Two scent structures (5–7 m apart) were used at each site, one on each side of a camera and distributed feed (silage), to increase the scent and decrease the effect of wind direction at the sites. Wooden structures of the exact same design but without pieces of fur were attached the same way at sites during control weeks. To maintain similar conditions each week, we exchanged all pelt pieces with fresh pieces for each treatment week, and scent structures were not mixed between treatments. Due to the cold temperatures during our experiment, pelt pieces did not rot. At each site, a remote camera (Scoutguard, model SG560C; HCO Outdoor Products, Norcross, CA) was mounted, directly facing the feed at a distance of 5–10 m. Cameras recorded a 30-sec video on detection (maximum detection range was 22 m) followed by a 2-min time lapse during which the camera could not be triggered. The time lapse setting decreased the chance of recording the same individual multiple times, and saved camera battery life. The camera was always positioned so that direct sunlight into the camera lens was avoided and feed centered in the pictures.

Feeding sites had similar forested habitat types (coniferous dominated) but varied in degree of habitat openness. Variation was mainly created by anthropogenic opening of the forests, such as creation of agricultural fields and clear-cutting practices. Therefore, some feeding sites were surrounded by closed forest, while others were closer to fields or other forest openings (see inset map in Fig. 1). To determine the effect of habitat openness on ungulate visitation, we measured sighting distance at each site using a red and white colored plank (180 cm high, 10 cm wide), which was placed at the feeding structure during measurements (see Ordiz et al. 2009). We measured the distance for the device to be completely hidden as we walked away from it in all four cardinal directions. The average of the four distances (i.e., sighting distance) was used in analyses (DePerno et al. 2003; Ordiz et al. 2009).

Statistical analyses

We used generalized linear mixed effect models in R (R Core Team 2012) using the MASS package (Ripley et al. 2014) with the quasipoisson family to model use of feeding sites in the study area. Moose and red deer numbers were omitted from all statistical modeling because of few records (N < 40). In addition to scent treatment, sighting distance was used as a covariate to investigate the effects of habitat openness at feeding sites and its interaction with the scent treatment. Due to the quasi-likelihood estimation, we were not able to use likelihood ratio tests for variables used in models. We assessed significant differences between scent treatments using multiple comparison tests (Tukey's) in the multicomp package (Holmström et al. 2008). We used the number of visits (i.e., the number of times the camera was triggered) as response variables in separate models for each ungulate species.

We modeled the number of visits on a weekly scale; because each site had a constant value of sighting distance (and each treatment lasted for 1 week). The number of visits at feeding sites was summed for each treatment week for each site. All models (three in total) included site and order of treatments as random effects to account for differences in variation among sites and to account for possible effects of treatment order by site.
Results

The total number of visits to sites varied among species but reflected the relative species abundances in the area: moose and red deer were the least frequent species, roe deer intermediate, and fallow deer and wild boar the most frequent species. All ungulates, except moose (only 14 visits), had more visits to control sites without scent than to brown bear and reindeer scent treatments (Table 1).

All species were affected by bear and reindeer scents to varying extents, either through lower number of visits or by altering use in response to habitat openness. Fallow deer strongly avoided bear scent compared to other scent treatments (Table 2). In addition, fallow deer showed a positive relationship between the number of visits and sighting distance at sites with bear scent, compared to control and reindeer scent sites where the relationship was negative (Fig. 2). Roe deer did not show clear responses to the introduced scents (Table 2); however, roe deer used sites with bear scent more if these sites were in more open areas (Fig. 2). Wild boar used sites exposed to bear scent less than sites without scent (Table 2) with a positive relationship between the number of visits and sighting distance at sites with bear scent, compared to control sites where the relationship was the opposite (Fig. 2).

Discussion

We determined that ungulates avoided predator scent and that perceived risk was mediated by habitat openness. Predator scent avoidance was particularly clear for fallow deer, both in terms of numbers of visits to sites and altered use of habitat openness. At a sighting distance of approximately 60 m the visitation in areas with predator scent approached that of control areas without scent. Our results indicate the human-created variation in habitat openness in forest landscapes creates opportunities for prey species to change their habitat selection to mediate predation risk. Studies in Bialowieża Primeval Forest (BPF), Poland, similar to the one we present here, did not detect an interaction between habitat openness and predator scent for red deer (Kuijper et al. 2014) and roe deer (Wiikénros et al. 2015). However, the sighting distance in BPF ranged from 5 to 20 m (comparing to 30–70 m in our study), which may be too narrow for ungulates to adjust their habitat selection to predation risk (Kuijper et al. 2013). Hence, instead of ungulates reducing their visitation rate to sites in dense habitats (as we showed in our human-modified system), ungulates in BPF increased their vigilance and reduced visitation duration to plots with carnivore scent. Whether ungulates select open or closed habitats in the presence of predators may depend on the hunting strategy of the predator (Thaker et al. 2011). Ambush predators are more likely to kill in denser habitat types (Lone et al. 2014), which may push prey into open habitat, whereas the risk of cursorial predators is higher in open areas, resulting in ungulates selecting denser cover (Cred et al. 2005). Brown bears are more likely to occupy relatively dense or rugged habitat (Martin et al. 2010; Ondž et al. 2011) and may hunt in an ambush-predatory manner (Garneau et al. 2007), which may be the reason why ungulates in our study reduced their use of sites with dense vegetation when bear scent was present. In addition to the aspect of how far ungulates can see at sites, scent cues may be stronger in denser sites due to less wind, which may intensify the

Table 1. Number of visits (i.e., number of recorded videos) to artificial feeding sites for five sympatric ungulate species in southeastern Sweden, March and April 2013. The number of visits for each species and treatment level represents the number of videos summed over 30 sites and 1 week of sampling per site (i.e., a total of 210 camera trapping days for each species and treatment level combination).

<table>
<thead>
<tr>
<th>Species</th>
<th>Scent treatment</th>
<th>Visits</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fallow deer</td>
<td>Brown bear</td>
<td>324</td>
</tr>
<tr>
<td></td>
<td>No scent (control)</td>
<td>462</td>
</tr>
<tr>
<td></td>
<td>Reindeer (control)</td>
<td>355</td>
</tr>
<tr>
<td>Moose</td>
<td>Brown bear</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>No scent (control)</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Reindeer (control)</td>
<td>7</td>
</tr>
<tr>
<td>Red deer</td>
<td>Brown bear</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>No scent (control)</td>
<td>37</td>
</tr>
<tr>
<td></td>
<td>Reindeer (control)</td>
<td>6</td>
</tr>
<tr>
<td>Roe deer</td>
<td>Brown bear</td>
<td>120</td>
</tr>
<tr>
<td></td>
<td>No scent (control)</td>
<td>196</td>
</tr>
<tr>
<td></td>
<td>Reindeer (control)</td>
<td>97</td>
</tr>
<tr>
<td>Wild boar</td>
<td>Brown bear</td>
<td>377</td>
</tr>
<tr>
<td></td>
<td>No scent (control)</td>
<td>393</td>
</tr>
<tr>
<td></td>
<td>Reindeer (control)</td>
<td>343</td>
</tr>
</tbody>
</table>

Table 2. Tukey’s multiple comparisons of model estimates for the frequency of ungulate visits to feeding sites with three different scent treatments, in southeastern Sweden, March and April 2013.

<table>
<thead>
<tr>
<th>Ungulate species</th>
<th>Treatment comparison</th>
<th>Estimate</th>
<th>SE</th>
<th>t-value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fallow deer</td>
<td>Control – Bear</td>
<td>3.601</td>
<td>1.194</td>
<td>3.015</td>
<td>0.007*</td>
</tr>
<tr>
<td></td>
<td>Reindeer – Bear</td>
<td>3.988</td>
<td>1.281</td>
<td>3.113</td>
<td>0.005*</td>
</tr>
<tr>
<td></td>
<td>Reindeer – Control</td>
<td>0.367</td>
<td>1.038</td>
<td>0.373</td>
<td>0.926</td>
</tr>
<tr>
<td>Roe deer</td>
<td>Control – Bear</td>
<td>3.542</td>
<td>1.352</td>
<td>2.681</td>
<td>0.143</td>
</tr>
<tr>
<td></td>
<td>Reindeer – Bear</td>
<td>3.074</td>
<td>1.527</td>
<td>2.013</td>
<td>0.108</td>
</tr>
<tr>
<td></td>
<td>Reindeer – Control</td>
<td>0.532</td>
<td>1.283</td>
<td>0.415</td>
<td>0.909</td>
</tr>
<tr>
<td>Wild boar</td>
<td>Control – Bear</td>
<td>2.155</td>
<td>0.911</td>
<td>2.385</td>
<td>0.047*</td>
</tr>
<tr>
<td></td>
<td>Reindeer – Bear</td>
<td>1.035</td>
<td>0.953</td>
<td>1.192</td>
<td>0.458</td>
</tr>
<tr>
<td></td>
<td>Reindeer – Control</td>
<td>–1.019</td>
<td>0.872</td>
<td>–1.169</td>
<td>0.471</td>
</tr>
</tbody>
</table>

*Significant pair-wise comparison with P < 0.05.
effect of the scent. It is possible that this aspect is important, especially for the more night active species such as fallow deer and wild boar, for which sight would potentially be less important. Habitat openness reflects risk and food availability, particularly for forest ungulates. In forest habitat, food availability is generally higher in forest gaps with increased light availability and forest ungulates preferentially feed in these forest gaps (Kuijper et al. 2009). Therefore, spatial distribution of food availability likely interacts with predation risk (Schmidt & Kuijper 2015). However, in our study design food availability was standardized across the habitat openness gradient through the provision of large amounts of feed at each site. Hence, food availability did not confound predation risk in our study.

Multipredator systems often include contrasting risks created by predators with different hunting techniques, suggesting that selection of more open habitat by prey to avoid one predator may actually increase risk created by another predator. For example, Løne et al. (2014) showed that roe deer live in a complex landscape of fear where predation risk by lynx was highest in closed habitats while...
human hunting created highest risk in open habitats. Importantly, the fact that, in our study, brown bear scent reduced prey species’ use of areas with denser vegetation may be maladaptive in areas with high human activity due to the increased risk of human-caused mortality in more open habitats (Lone et al. 2014). However, the role of human activity is complex, as many studies have shown that human settlements may act as refuge areas where ungulates find protection from large carnivores (the so-called “human shield” effect, Berger 2007). Future research on the nature of contrasting risk effects between different predatory types (e.g., ambush vs. chasing) and large carnivores and humans in human-modified landscapes is imperative, because most wildlife populations reside in, or depend on, regions outside protected areas. Roe deer and wild boar did not show as clear responses to avoid predator scent as fallow deer. Generally, wild boars are relatively unresponsive to predation risk, likely because they are a primary prey species of European large carnivores (Kuijper et al. 2014; Wikenros et al. 2015). The lack of a strong response by roe deer is more difficult to explain but may be partly due to our relatively small sample size for this species (only 1/3 of the sample size of fallow deer). However, the effect of habitat openness was similar for all species. Therefore, our results indicate that prey naïveté is not present in our study area. This reflects other recent studies that showed that prey maintained antipredator responses to their predators that went extinct over a century ago (Li et al. 2011; Chamaillé-Jammes et al. 2014). The main explanation for prey maintaining these responses is that the innate mechanism of recognition of dangerous predators is an evolutionary response (Chamaillé-Jammes et al. 2014). Traits with simple reaction norms and direct effects on survival are likely to experience strong, directional selection which may effectively drive the trait to fixation in a population, explaining why it is retained even in the absence of selective pressure. Indeed, Ferrero et al. (2011) recently showed that, contrary to noncarnivores, a large range of carnivores produce large amounts of the exact same chemical in their urine and that this chemical elicits antipredator responses in prey. Predator avoidance by prey species without prior experience to the predator is important, because innate antipredator responses will likely reduce the risk of prey populations suffer from high predation rates due to prey naïveté if predators return (Berger et al. 2001).

Innate responses to novel scents, not just carnivore scents, may have been beneficial during the course of evolution (Barks and Godin 2013). To control for the possible effects of novel scent we added reindeer scent as additional “novel scent” control. Without such control scents, prey responses to introduced carnivore scents could simply be due to the novelty and not the actual carnivore cue. However, we did not detect a clear response to reindeer scent in our study area. Nevertheless, we stress the importance of including nonpredator control scents when studying prey response to predator cues to avoid overestimating risk effects on prey, particularly when looking at effects of locally extinct, or recently recolonizing carnivores.

In conclusion, we have experimentally demonstrated that ungulates reduce their visitation of forest habitats with signs of recent predator presence but that the strength of this response declines with increasing human-created openness of the forest habitat. Human alterations to forested landscapes allow ungulates to change habitat selection in ways that are not possible in undisturbed forests. Interestingly, however, predators will likely use the altered landscape heterogeneity to their advantage. Thus, predator–prey interactions may develop in human-modified landscapes in novel directions that are yet to be explored, which is highly relevant when multiple large carnivore species are recolonizing former ranges that are now heavily impacted upon by humans (Chapron et al. 2014). In many regions, it is often the carnivore and not the prey that is of conservation concern; however, any negative effect on popular game species can be crucial for the public acceptance of large carnivores (Roskaft et al. 2007; Gangaa et al. 2013). Understanding risk behavior in pristine environments is important to assess what we are potentially losing when natural ecosystems are affected by anthropogenic change. However, future research should focus on human-modified regions to understand the predator–prey interactions actually present in these landscapes. The current wildlife comeback in Europe makes this particularly relevant.

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Data Accessibility
Data will be archived in the public archive Dryad (http://datadryad.org/).

Conflict of Interest
None declared.

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Swedish Environmental Protection Agency (SEPA) 2015. Available at http://www.naturvardsverket.se/SA-mat-miljon/
CHAPTER 2

Brown bears evoke a three-dimensional landscape of fear

Abstract

Predation risk varies spatiotemporally across many landscapes and the behavioral changes made by prey to cope with such variation in risk can lead to large cascading impacts on vegetation. While several studies have investigated the impact of predators on ungulate foraging decisions across the two-dimensional plane, few studies have considered how predation risk affects ungulate foraging decisions in a three-dimensional landscape of fear, where height-specific browsing may be affected by perceived risk with resulting consequences for plant recruitment. To test this, we quantified ungulate browsing in plots with varying levels of risk (assessed by brown bear habitat selection). In each plot we counted browsed and unbrowsed twigs in five height classes and collected data on other habitat related variables (e.g., habitat openness, escape impediments, twig height, and distance to roads). We measured the height of the tallest sapling in the plot, to document potential effects on tree recruitment. We found that the probability of browsing was lower in areas that were more intensively used by brown bears, especially where visibility was low. Patterns in tree recruitment followed those of browsing, with the height of the tallest sapling being shorter in low risk areas, where the probability of browsing was higher. Furthermore, ungulates browsed at lower heights where risk was higher, suggesting that predation risk by brown bears pushed browsing downwards, potentially because of an increased vulnerability when reaching twigs at higher tree heights. Our results suggest that predation risk by brown bears may release vegetation from browsing pressure, with implications for tree recruitment.
as trees may be released from extensive browsing (browse traps) and reach a ‘safe’ height sooner in areas with predators.

**Key Words** *Alces alces*, browsing, landscape of fear, large carnivores, predation, trophic cascades, antipredator behavior, *Ursus arctos*

**Introduction**

Predators affect ungulate populations directly (e.g. killing individuals) and indirectly (e.g. imposing risk) (Creel et al. 2014). Predation risk varies spatiotemporally across the landscape and depends on predator and prey characteristics, as well as on landscape features, creating a so-called landscape of fear (Laundré et al. 2001). Landscape characteristics such as terrain ruggedness and vegetation structure can impede or facilitate hunting success for predators, as well as impede or facilitate cover or escape for prey. For example, in the presence of wolves *Canis lupus* (cursorial predators), elk *Cervus elaphus* and moose *Alces alces* use denser habitats to avoid predation (Kunkel and Pletscher 2000, Creel et al. 2005). Conversely, vegetation density facilitates lynx *Lynx lynx* (ambush predators) predation on roe deer *Capreolus capreolus* (Lone et al. 2014). Variation in risk intensity can occur at very fine scales, where prey respond to risk at the level of foraging patches as small as a few square meters because of the presence of structural escape impediments (e.g., large tree stumps or boulders) (Kuijper et al. 2014). Thus, antipredator responses may occur on many scales of habitat selection (Johnson 1980, Kunkel and Pletscher 2000), from overall habitat selection (home range scale) down to specific patches (feeding site scale) (Johnson 1980, Ripple and Beschta 2007, Kuijper et al. 2014) or even the trees in those patches (food item scale) (Johnson 1980, Danell et al. 1991, Faber and Lavsund 1999). Moreover, several studies have
indicated that variation in risk across landscapes can lead to cascading effects on lower trophic levels (Ripple and Beschta 2007, Ford et al. 2014).

The landscape of fear concept has been successfully applied to a two-dimensional context, where ungulates respond to variation in risk across a two-dimensional plane. For grazing ungulates, which mostly forage on monocotyledons (such as grasses) at ground level, this may work well. However, for browsers, which feed on dicotyledons (such as shoots from woody plants) at various heights, there is an additional dimension of resource selection (du Toit and Olff 2014). Preferred browsing height varies among the browser species and may depend on woody plant species and the location of nutritious shoots (du Toit and Olff 2014, Nichols et al. 2015). Browsing height may further be affected by predation risk, as different feeding postures affect vigilance and escape opportunities (Cameron and du Toit 2005). It has been hypothesized that browsing at plant heights that are high relative to browser shoulder height is more risky than browsing at lower plant heights (Cameron and du Toit 2005), and that foraging at ground level may be risky as it may obstruct the view (Altendorf et al. 2001). Hence, predators can release vegetation from browsing pressure in the xy-plane of the landscape, and may further affect browsing vertically (z-axis) (i.e., tree height specific browsing). However, we are not aware of any empirical tests that link browsing height to levels of predation risk.

Browsing pressure can impact tree recruitment (Belsky 1984, Kuijper et al. 2010) by leading to demographic bottlenecks for plants, where browsing prevents plants to transition from one height class to the next (Staver et al. 2009). Under high browsing pressure, trees and shrubs can become trapped in a ‘browse trap’ (Sankaran et al. 2013), from which they
cannot escape under the ongoing browsing pressure. In its extreme form, browsing may lead to browsing lawns where all woody plants are trapped in a short state that is continuously browsed (Cromsigt and Kuijper 2011). As mentioned, predators have the potential to release plants from such limitations by affecting ungulate browsing height and browsing intensity throughout the landscape.

Brown bears *Ursus arctos* can have large negative impacts on ungulate neonate survival (Zager and Beecham 2006, Swenson et al. 2007). In the Swedish taiga, brown bears heavily predate on moose calves during the moose calving season (May - June) (Rauset et al. 2012) and on average, seven moose calves (< 1 month of age) are killed per bear in central Sweden, with some bears observed killing up to 14 calves in less than a month (Rauset et al. 2012). Annually, brown bears kill approximately 26% of all moose calves in southcentral Sweden (Swenson et al. 2007). Although bears target mostly calves, adult moose (especially females) are occasionally preyed upon (Dahle et al. 2013). Research has shown that mothers are highly aware of factors influencing risk both for themselves and for their calves, and adjust their behavior according to the level of risk (White and Berger 2001). Further, it is possible that this risk avoidance is influencing and enhancing future risk-aversive behavior of young individuals (Galef and Laland 2005). Therefore, brown bears are likely to affect moose foraging behavior, and particularly during the calving season, when calves and their mothers are most vulnerable to predation. Moreover, these behaviorally mediated effects of brown bear predation may cause cascading impacts on vegetation.

In this study, our objectives were to evaluate moose browsing on woody plants in relation to brown bear habitat selection (hereafter referred to as risk). We quantified moose browsing
in three-dimensional space by counting browsed and unbrowsed twigs at different height classes in plots, stratified over varying levels of risk. Quantifying browsing behavior provided the opportunity to study moose behavior and vigilance in retrospect, without disturbing moose when they forage in risky landscapes. Specifically, our objectives were to determine if; the probability of moose browsing declines with increasing predation risk, if moose browse at different heights in areas with low versus high risk, and if there are resulting impacts on tree recruitment. We also included additional factors; horizontal cover and escape impediments, which mediate risk and may impact browsing behavior.

Methods

Study area

The study was conducted in the counties of Dalarna and Gävleborg in south-central Sweden (~61°N, 15°E), a topographically diverse landscape dominated by Scots pine *Pinus sylvestris* and Norway spruce *Picea abies* forests. Deciduous trees were mainly birch *Betula pendula* and *B. pubescens*, rowan *Sorbus aucuparia*, aspen *Populus tremula* and willows *Salix* spp. occurring mostly in early successional stages on plantations, near bogs, or as scattered single trees in the otherwise pine and spruce-dominated commercial forests. The study area is within the larger study area of the Scandinavian Brown Bear Research Project (SBBRP), and is one of the highest density brown bear areas in the country. Moose and brown bear densities were estimated to 9.20/10 km² (Cederlund and Wallin 1998) and 0.3/10 km² (Solberg et al. 2006), respectively. Roe deer generally occur at low densities throughout this region (Zimmermann et al. 2015). Wolves were near absent in the study area during the study period. Both moose and brown bears are hunted in the area, with potential impacts on
behavior (see e.g. Steyaert et al. 2016). The road network is well developed, with many small gravel logging roads. Hunting cabins, summer houses, and smaller communities were distributed throughout the study area.

Data collection

We divided the study area into low, medium, and high risk based on probabilities of bear habitat selection, using a resource selection map from the SBBRP based on Steyaert et al. (2013). Increased brown bear habitat selection is likely to increase risk for ungulates as brown bear are generally opportunistic hunters (Bastille-Rousseau et al. 2011). The 50*50 m pixel resource selection map was based on habitat selection of 51 radio-collared bears throughout brown bear mating season (1 May - 15 July) during 2006 - 2010 (Steyaert et al. 2013), which is also the time when brown bears heavily predate on moose. Variables included to predict bear habitat selection were human disturbance, Normalized Difference Vegetation Index, terrain characteristics, and land cover type (Steyaert et al. 2013). We distributed study sites in areas of low and high probability of bear habitat selection (11 sites in high and 10 in low, Figure 1). Low vs. high bear habitat selection was assigned by dividing the bear data into three classes, where sites were distributed in the lowest and highest levels of use to ascertain sampling in areas with contrasting levels of risk. Within each site, we distributed 20 plots with a 3 m radius on the nodes of a grid with an edge length of 100 m. We excluded plots without available browse species, or that were not meaningful to include for other reasons (e.g., recent thinning or clearcutting at the location). Tree and shrub species browsed were rowan, downy and silver birch, willow, and to a lesser extent alder Alnus glutinosa, pine, and juniper Juniperus communis. Spruce was common but was
not browsed in any of the plots, thus not included as an available browse species in this study.

Within each retained plot, we counted the number of browsed and unbrowsed apical and lateral shoots from the current year’s growing season in five height classes (0-50 cm, 50-100 cm, 100-150 cm, 150-200 cm, and 200-300 cm). We also measured the height of the tallest sapling in the plot as a proxy of tree recruitment. We calculated selection ratios by dividing the proportion browsed twigs by the proportion of available twigs (browsed + unbrowsed) for each height class within each plot. We measured sighting distance in four cardinal directions following (Ordiz et al. 2009). A sighting distance device was placed in the center of the plot and was walked away from until it was no longer visible behind vegetation or terrain (Ordiz et al. 2009). The mean of the four measured distances was used in analyses. We counted potential escape impediments (large rocks > 30 cm high/wide, stumps > 30 cm high/wide, or coarse woody debris CWD > 1 m long and 15 cm in diameter) within 20 m from the plot center. We further assessed general habitat type and classified it into young regenerating or older forest. We measured distance to nearest road from each plot center in ArcMap (ESRI 2011). We used cumulative primary productivity measurements for the location of each plot to control for effects of productivity on browsing and sapling height in plots. Median productivity values (MODIS Fpar greeness) were summed for each 8 day period from 2003-2014 (see Coops et al. 2008).

Statistical analyses

We used generalized linear mixed effect models in R (R Development Core Team 2010) and the package MASS (Venables and Ripley 2002) for the three different response variables we
investigated; i.e., the probability of browsing per plot, the height of the tallest sapling in each plot, and the selection ratios for the different height classes in each plot (Table 1). We used the quasibinomial family when analyzing the probability of browsing to correct for overdispersion (residual deviance > residual degrees of freedom). In addition to the intensity of bear habitat selection (covariate), we considered several biologically relevant explanatory variables in our models (e.g., sighting distance, escape impediments, productivity, browsing height class, and relevant interactions) (Table 1). We included ‘site’ as a random intercept in all models, and nested plot within site for the height selection models. Because our aim was to explain which factors had an impact on browsing behavior and sapling height, and because Akaike’s information criterion is not defined for quasibinomial models, we used a stepwise backward model selection procedure, in which we systematically removed insignificant terms (Shmueli 2010, Crawley 2013) with an alpha level of 0.05. We evaluated model fit by plotting residuals vs. fitted values.

**Results**

In total, 271 plots in 21 sites were quantified in terms of browsing. The final model to assess the probability of browsing in plots included sighting distance, bear habitat selection, distance to the nearest road, escape impediments, and the interaction between sighting distance and bear habitat selection (Table 2). The probability of twigs being browsed was lower in areas with higher levels of risk (Figure 2, Table 2). Likewise, browsing probability was generally lower in the more open areas; however, the probability increased with sighting distance where predation risk was higher (Figure 2 and Table 2). Probability of browsing
declined with increasing amount of potential escape impediments, although this effect did not depend on bear use. Browsing probability was generally higher closer to roads (Table 2).

The final model to assess the height of the tallest sapling per plot included sighting distance, habitat type, bear habitat selection, and the interaction between sighting distance and bear habitat selection (Table 3). Overall, the height of the tallest sapling in the plot was higher in the more open areas, but the opposite was found where brown bear risk was high (Figure 3, Table 3). Saplings were on average ~20 cm taller in the younger, regenerating habitat types compared to older ones (irrespective of risk).

At the finest scale, looking at the selection ratios for browsing at different heights at the plot level, browsing height and probability of bear use and their interaction were retained in the final model. The interaction between browsing height and bear use was negative (i.e., there was a stronger selection for twigs at lower heights in areas with higher probabilities of bear habitat selection; $\beta = -0.019$, standard error = 0.003, df = 363, t-value = -5.52, and $p = 0.000$ (Figure 4), and the most selected foraging height decreased by two height classes in these areas (Figure 4C-D).

**Discussion**

When exposed to predators, prey are likely to exhibit antipredator responses that minimize the risk of injury or death (Sih et al. 2010). As expected, moose in our study area responded to brown bear predation risk by foraging less in areas with higher intensities of brown bear habitat selection. The few studies that have investigated the effects of brown bear risk on the behavior of moose or other ungulates corroborate our results (Berger et al. 2001, Berger 2007, Sahlén et al. 2016). Further, this study is among the first to demonstrate the height-
wise foraging decisions that ungulates make in a three-dimensional landscape of fear. Complimentary to previous studies, we noted that bear predation risk on moose may have trophic cascading effects, with saplings being taller in areas with higher predation risk. Our results indicate that trees can more easily escape browse traps in areas that are more risky for moose. Although trait-mediated trophic cascades have been described previously (Pace et al. 1999, Ripple and Beschta 2007) we are not aware of any studies investigating the effects of brown bears in terms of their potential cascading effects on the vegetation through trait-mediated effects.

Moose are not only selective at the scales of landscapes, forest stands and individual trees (Bergqvist et al. 2003), but also at the within–tree scale (Danell et al. 1991). Browsing height in plots depended on brown bear presence, which is an additional dimension to the landscape of fear concept that we currently know very little about. In a study area in southern Sweden, Nichols et al. (2015) noted that moose browsed at a variety of heights, with an average height of 120 cm. In our study, moose generally browsed above 150 cm in the low bear use areas, but shifted towards lower heights in the high bear use areas. Moose have been documented to extensively browse the top terminal shoot on trees in areas with low large carnivore densities (Faber and Lavsund 1999). Twigs are generally more nutritious and protein-rich higher up, as actively growing newer shoots (du Toit 1990, Faber and Lavsund 1999); thus, without risk, browsers are indeed expected to feed at higher heights. In areas with predators, feeding above ground level may also increase the ability to scan the surroundings (Altendorf et al. 2001). However, as suggested for other ungulates, feeding at higher heights might not be entirely beneficial, as it entails adopting a vulnerable posture (du Toit 1990). Vigilance may be
reduced when the head is directed upwards, and standing closer to the tree may impede escape for moose, as suggested for giraffes *Giraffa camelopardalis* (du Toit 1990). Maybe even more important, foraging at higher heights may increase handling time as it takes more effort and demands more focus to pull off less steady twigs higher up. Browsing at different heights generally occurred according to availability in areas with high intensity of bear use, which was expected as prey species are predicted to forage less selectively and more according to availability under risk. In theory, each effort to avoid or select food items in a specific patch cost additional time and energy which eventually can cost prey individuals their life (Kie 1999).

Browsing may impose height-structured recruitment limitations, or demographic bottlenecks, on trees (i.e. a ‘browse trap’) (Sankaran et al. 2013, Staver and Bond 2014). Our results suggest that brown bears have the potential to release the vegetation from browsing in areas that ungulates perceive risky, not only by altering browsing intensity, but also by shifting browsing height. Therefore, the risk of being browsed for a plant individual at a certain height not only depends on the ungulate species in the landscape, but also on the presence of predators. As mentioned, predators may have the potential to release plants from demographic bottlenecks by reducing browsing pressure and push ungulate browsing to lower heights. The latter would mean that individual tree saplings may generally escape the browse trap at lower heights and more quickly escape the demographic bottleneck if predators are present. In addition, habitat openness may affect these processes, adding further complexity to the three-dimensional landscape of fear.
Indeed, our results indicate that perceived risk was mediated by the openness in the surroundings, which is in line with earlier investigations of this model system (Sahlén et al. 2016). Brown bears are generally predisposed to use rather dense vegetation in Scandinavia (Martin et al. 2010, Steyaert et al. 2013) and are more of an ambush, or opportunistic predator than cursorial (Garneau et al. 2007, Bastille-Rousseau et al. 2011). Thus, moose may be relatively safe while foraging in open forest stands, even in areas where brown bear use is generally high, because moose have a greater chance to detect and escape an approaching bear. These patches may be important for moose throughout the landscape, especially for females and their calves, which are the most targeted groups by brown bears (Swenson et al. 2007, Dahle et al. 2013). We further suggest that humans have the potential to affect predator-prey interactions in areas with intensive forestry, by altering habitat openness on a patch-scale throughout the landscape.

We determined that antipredator responses will likely have an effect on the regeneration of trees in our study area. However, whether these effects are biologically important remains unclear. Over time and in undisturbed areas, cascading effects may have large impacts on the vegetation structure (Pace et al. 1999); yet, few areas are undisturbed over longer time frames whether the disturbance is human caused (e.g., thinning, logging, soil scarification, or extensive human activity) or natural (e.g., fire, storms, or landslides). In boreal forests natural disturbance events have a relatively low frequency of occurrence. However, our study area is intensively affected by the forestry industry, and it is unlikely that cascading effects by an apex predator would considerably affect the vegetation over time, as the forest is dominated by severe ground preparation and clearcutting throughout the study area (every 80 years on
average) (Kuuluvainen et al. 2012). Thus, humans may dilute traces of cascading effects in areas where these could potentially have an impact. Notably, humans also affect both ungulate and brown bear behavior (Neumann 2009, Ordiz et al. 2011). However, human risk is limited to daytime and hunting is restricted to the hunting season, whereas brown bear risk is rather constant. Although humans may be the most important predators in this study area overall, we are rather confident that our results reflect the risk that brown bears pose on ungulate behavior, especially as we quantified early summer browsing when predation by brown bears is at its peak, and human recreational use is rather low (no hunting, mushroom or berry picking).

Moose may not have been the sole browser species in plots in this study, although they were by far the most dominant. Whereas it was relatively easy to distinguish hare *Lepus* spp. browse bites from ungulate browse bites in the field, bites from different ungulate species is nearly impossible to discriminate between. However, roe deer (the only sympatric ungulate species in this area) do generally not browse above 1 m and occurred in low densities. Even if roe deer browsing would have been more widespread than we would expect, the overall results that ungulates change browsing patterns depending on risk would still be relevant.

To conclude, we noted that brown bears affected ungulate browsing behavior and vegetation in an anthropogenic landscape. Browsing intensity and browsing height changed in response to predation risk, which suggest brown bears have the potential to affect lower trophic levels and release vegetation from browsing pressure in certain areas depending on the nature of the landscape of fear. Humans often alter the environments where predator-prey interactions occur (e.g., strongly affect habitat openness) (Kuijper et al. 2014, Ripple et al.
2014), and therefore it is also our responsibility to discover potential consequences this might have. One of the first steps is to increase our understanding of predator-prey interactions in areas where human activity occur. Because humans are not likely to stop the encroachment on wildlife habitat anytime soon, studies in anthropogenic landscapes are important to understand how to best manage and ameliorate our potential impacts.

Acknowledgments

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Bastille-Rousseau, G., D. Fortin, C. Dussault, R. Courtois, and J. P. Ouellet. 2011. Foraging strategies by omnivores: are black bears actively searching for ungulate neonates or are they simply opportunistic predators? Ecography 34:588-596.


Table 1. Variables used in modeling moose browsing in central Sweden, summer 2013.

<table>
<thead>
<tr>
<th>Response variables</th>
<th>Proportion browsed in plot</th>
<th>Height of the tallest sapling</th>
<th>Height selection ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fixed effects</td>
<td>Bear use</td>
<td>Bear use</td>
<td>Bear use</td>
</tr>
<tr>
<td></td>
<td>Sighting distance</td>
<td>Sighting distance</td>
<td>Sighting distance</td>
</tr>
<tr>
<td></td>
<td>Escape impediments</td>
<td>Escape impediments</td>
<td>Escape impediments</td>
</tr>
<tr>
<td></td>
<td>Productivity</td>
<td>Productivity</td>
<td>Height class</td>
</tr>
<tr>
<td></td>
<td>Habitat</td>
<td>Habitat</td>
<td>Bear use*Height class</td>
</tr>
<tr>
<td></td>
<td>Distance to road</td>
<td>Distance to road</td>
<td>Bear use<em>Height class</em>Sighting distance</td>
</tr>
<tr>
<td></td>
<td>Bear use*Sighting distance</td>
<td>Bear use*Sighting distance</td>
<td>Bear use<em>Height class</em>Escape imp.</td>
</tr>
<tr>
<td></td>
<td>Bear use*Escape impediments</td>
<td>Bear use*Escape impediments</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Bear use*Productivity</td>
<td>Bear use*Productivity</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Bear use*Habitat</td>
<td>Bear use*Habitat</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Bear use*Dist. to road</td>
<td>Bear use*Dist. to road</td>
<td></td>
</tr>
<tr>
<td>Random effects</td>
<td>Site</td>
<td>Site</td>
<td>Site/Plot</td>
</tr>
<tr>
<td>Distribution</td>
<td>Quasibinomial</td>
<td>Gaussian</td>
<td>Gaussian</td>
</tr>
</tbody>
</table>

Table 2. Parameter estimates (β), standard error (se), test statistics (t-value) and p-values for variables retained in the minimum adequate mixed effect quasibinomial model to explain the probability of ungulate browsing in survey plots in central Sweden, summer 2013.

<table>
<thead>
<tr>
<th>Variables</th>
<th>β</th>
<th>se</th>
<th>t-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Intercept)</td>
<td>2.529</td>
<td>1.072</td>
<td>2.358</td>
<td>0.0191</td>
</tr>
<tr>
<td>Sighting distance</td>
<td>-0.048</td>
<td>0.013</td>
<td>-3.889</td>
<td>0.0001</td>
</tr>
<tr>
<td>Bear use</td>
<td>-5.772</td>
<td>1.565</td>
<td>-3.688</td>
<td>0.0003</td>
</tr>
<tr>
<td>Distance to road</td>
<td>-0.002</td>
<td>0.001</td>
<td>-4.157</td>
<td>0.0000</td>
</tr>
<tr>
<td>Escape impediments</td>
<td>-0.022</td>
<td>0.011</td>
<td>-1.956</td>
<td>0.0516</td>
</tr>
<tr>
<td>Sighting dist.*Bear use</td>
<td>0.083</td>
<td>0.021</td>
<td>3.992</td>
<td>0.0001</td>
</tr>
</tbody>
</table>
Table 3. Parameter estimates ($\beta$), standard error (se), test statistics (t-value) and p-values for variables retained in the minimum adequate mixed effect gaussian model to explain the height of the tallest sapling in survey plots in central Sweden, summer 2013. The reference level for comparison for the categorical variable ‘Habitat’ is ‘Older forest’.

<table>
<thead>
<tr>
<th>Variables</th>
<th>$\beta$</th>
<th>se</th>
<th>t-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Intercept)</td>
<td>36.849</td>
<td>45.420</td>
<td>0.811</td>
<td>0.4181</td>
</tr>
<tr>
<td>Sighting distance</td>
<td>1.756</td>
<td>0.708</td>
<td>2.481</td>
<td>0.0139</td>
</tr>
<tr>
<td>Habitat</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Young regenerating forest</td>
<td>23.586</td>
<td>8.695</td>
<td>2.713</td>
<td>0.0072</td>
</tr>
<tr>
<td>Bear use</td>
<td>141.401</td>
<td>66.324</td>
<td>2.132</td>
<td>0.0341</td>
</tr>
<tr>
<td>Sighting dist.*Bear use</td>
<td>-3.274</td>
<td>1.124</td>
<td>-2.914</td>
<td>0.0039</td>
</tr>
</tbody>
</table>
Figure 1. Study area and sites (black squares) for quantifying ungulate browsing in areas with different intensity of brown bear habitat selection, central Sweden, summer 2013. (RSF map created by Steyaert et al. 2013.)
Figure 2. Relationship between the probability of browsing, intensity of brown bear use (divided into low [left] and high [right] for graphics) and sighting distance for plots in central Sweden, summer 2013.
Figure 3. Relationship between the height of the tallest sapling in the plot, intensity of brown bear use (divided into low [left] and high [right] for graphics), and sighting distance in central Sweden, summer 2013.
Figure 4. Selection ratios (proportion browsed / proportion available twigs) (A and B), total number of browsed twigs (C and D), and the availability of twigs (E and F), for areas with different intensity of brown bear use (divided into low [left] and high [right] for graphics) in central Sweden, summer 2013.
CHAPTER 3

Large-scale spatial variation in long-term stress hormone levels in moose

Abstract

Stress is an evolutionary trait that allows organisms to respond to challenges. The physiological consequences of short-term stress responses lead to increased individual survival as it prepares the body for fight or flight through catabolic reactions in the body. However, long-term stress is shown to negatively impact growth, immune system, reproduction, and survival, with implications for the management and conservation of wildlife populations. Yet, relatively little is known about the impact of stress in wildlife or the factors contributing to increased long-term stress levels. One method to measure long-term stress in mammals is to quantify slowly incorporated stress hormone (cortisol) in their hair, which most likely reflect a long-term average of their stress responses. In this study, we sampled moose *Alces alces* across the Swedish distribution to study the relative effect of landscape variables and disturbances on moose hair cortisol levels. We included variables related to human disturbance, ungulate competition, large carnivore density, and ambient temperature. The most important variable explaining the variation in hair cortisol levels in moose was the long-term average temperature sum in the area where they lived, with higher hair cortisol levels in the south where temperatures are higher. Hair cortisol levels were also positively associated with road density; and the closer to towns and cities the moose lived, the stronger effect of roads. In contrast, large carnivore occurrence was associated with lower levels of hair cortisol in moose, indicating a lower stress load in these areas, potentially because large carnivore habitat use is higher in areas away from human disturbance. To
mitigate a potential negative impact from warming temperatures and human activity on moose, one way is to increase the availability of old growth forests, as these are cooler and are likely to have lower levels of human disturbances.

**Key Words** *Alces alces*, hair cortisol, human disturbances, human-modified landscapes, moose, predation risk, Scandinavia, stress

**Introduction**

Long-term stress affects individual fitness (Boonstra 1998, Blas et al. 2007), with potential implications for the performance of wild populations. While short-term stress may allow individuals to perform better in emergency situations such as the imminent threat of predation or physical conflict, this is not the case for long-term stress. In contrast, the physiological consequences of long-term stress include reduced fertility (Strasser and Heath 2013), cognition (McEwen and Sapolsky 1995), immune system (Khansari et al. 1990), body condition and survival (McEwen 1998). Despite this overarching importance of stress for individual and population performance, relatively little is known about the physiological effects of environmental stressors in wild populations.

Long-term, or chronic, stress may be manifested in a population as increased rates of disease or decreasing population growth rates (Wikelski and Cooke 2006), but these trends may be masked by intense harvest or mistaken for density dependent processes. Because changes in underlying vital rates can have direct effects on population dynamics and viability, understanding the role of stress for these processes in wild populations is important. This is particularly true for slowly reproducing species or small populations. As there is often a time lag between disturbance events and the associated population decline, real-time data to
monitor relative stress levels could provide an early warning system of changes that affect populations (Bourbonnais et al. 2013).

Multiple factors may affect stress levels and individual health negatively, including; fasting (Ward et al. 1992); habitat fragmentation (Ewers and Didham 2006); human activity (Bourbonnais et al. 2013), disease, injuries, discomfort, or pain (Vachon-Presseau et al. 2013); climatic shifts and heat (Silanikove 2000, Bechshoft et al. 2013); predation risk (Boonstra 1998, Creel et al. 2002); competition (Fey and Trillmich 2008); and displacement (Davenport et al. 2006). For example, Bechshøft et al. (2013) noted that polar bear *Ursus maritimus* stress levels vary with fluctuations in North Atlantic Oscillation (temperature) and ice cover, and Boonstra et al. (1998) noted that predation levels affected stress levels in snowshoe hares *Lepus americanus*. Notably, there may be synergistic effects of stressors occurring across the landscape, and the frequency and magnitude of these may determine the ultimate allostatic load (i.e., the physiological consequences of long-lasting exposure to repeated or chronic stress) on an individual.

Specifically, species living in anthropogenic landscapes are influenced by the activities of humans in many ways. Humans impact land use, fragmentation, and impose risk through e.g. hunting (Lone et al. 2014). One profound effect, although still rather abstract in most regions, is the human influence on Earth’s increasing average surface temperature (IPCC 2008). Consequently, some species are now dealing with increasing temperatures in areas greatly affected by humans, which may have further negative impacts on health and reproduction, part of it from increased stress responses.
Cortisol is a hormone involved in a wide range of physiological processes such as immunity, digestion, reproduction, and growth (Russell et al. 2011). It is used as a biomarker of stress in humans and other vertebrates (Sheriff et al. 2011). Growing hair incorporates unbound and potent cortisol molecules circulating in the bloodstream; thus, the amount of cortisol extracted from hair is commonly used to assess a long-term average of the systemic exposure to cortisol (Russell et al. 2012). Cortisol levels in hair hence offer a long measurement (e.g., spanning over weeks or months) of overall stress load, and has been used in many studies investigating long-term stress levels in a variety of mammals (Macbeth et al. 2010, Ashley et al. 2011, Meyer and Novak 2012, Weed et al. 2012).

Although many studies have investigated the relationship between stress levels and specific variables such as predation risk or displacement, there is currently a lack of knowledge about the effects of several landscape variables on long-term stress in wildlife (but see Bourbonnais et al. 2013). To study how long-term cortisol levels vary on a landscape scale requires many sampled individuals across gradients of the landscape variables of interest (Wingfield et al. 2013). Here, we explore large-scale patterns of hair cortisol levels in a solitary ungulate, moose *Alces alces*, across a 2000 km latitudinal gradient.

We use moose as a study species because they are widely distributed across Sweden, thereby occurring along gradients of anthropogenic activities, carnivore distributions, climate, and sympatric ungulate species. Being a ruminant adapted to colder climates moose are especially sensitive to warm temperatures (Renecker and Hudson 1986), which makes them a good model species investigating temperature effects on stress levels. Declining moose numbers have been observed across the southern ranges of their distribution in North
America (Murray et al. 2006, Lenarz et al. 2009, Lenarz et al. 2010). The decline has been suggested to be partly caused by a variety of climate-related stressors, including higher average annual temperatures, long strings of mild winters, and increasingly favorable conditions for ticks, parasites and other invasive species (Murray et al. 2006, Lenarz et al. 2010). Also in Sweden, moose in the southern ranges are more exposed to less favorable conditions in terms of higher temperatures and increasing prevalence of parasites (Valimaki et al. 2010).

We sampled moose individuals and investigated the relative impact of several landscape variables on hair cortisol levels in Sweden. We examined how biological (intrinsic) and environmental (extrinsic) factors, including long-term temperature variation and risk, correlated to the variation in hair cortisol levels in moose, and determined whether reported long-term injured or emaciated individuals had higher hair cortisol levels than healthy individuals, which would be expected if hair cortisol is reflecting allostatic load.

**Methods**

**Study region**

Sweden has climatic gradients occurring from the Scandic mountains in the west to the Baltic sea in the east, and from Arctic tundra in the north to boreal and temperate broad-leaved forests in the south. Scandinavia is predicted to have a faster rate of increase in temperature than many other places in the world (SMHI 2014), and climate gradients correlate closely with human density (higher in the south). Further north, moose are more disposed to migrate considerable distances, from cooler summer ranges in the mountains to milder winter ranges towards the coast, compared to the south where they are more stationary (Singh et al. 2012).
In Sweden, moose is a highly important species; moose is a national symbol that generates tourism, it is Sweden’s most important game species, but it is also involved in over 10% of the wildlife-vehicle collisions (Viltolycksstatistik 2012), and a nuisance animal for the forest industry. The moose hunt is one of the most extensive harvests in Scandinavia and approximately 100,000 (from a population of ~350,000 individuals) are shot yearly. With the hunting comes traditions and cultures that often clash with the increasing or expanding populations of brown bear *Ursus arctos* and grey wolf *Canis lupus*.

Historically, large carnivores were abundant throughout Sweden. However, all populations were close to extinction due to eradication campaigns ending in the 1940s. Today, populations of brown bear and wolf are regulated by licensed hunts and the removal of the occasional problem animal. With populations generally stable or increasing, wolf and brown bear occur mainly in central Sweden and to the western part of the country. Sympatric ungulate species are roe deer *Capreolus capreolus*, reindeer *Rangifer tarandus* (domestic), fallow deer *Dama dama*, red deer *Cervus elaphus*, and wild boar *Sus scrofa*.

*Sample collection*

Hair sampling was conducted by hunters during the moose hunt in fall and winter 2012. Sampling protocols were distributed to hunters for hair sampling. Hair was collected by cutting the hair as close to the skin as possible using a clean knife or electric clipper over an area of approximately 4 cm². Samples were collected from three different parts of the moose; however, to standardize the methods only samples from one part (the rump) was analyzed in this study. Also, we asked hunters to document the GPS-location of the kill site, general health status and older injuries of the moose, its sex/reproductive status, and the hunting
method by which it was killed. Of the 1000 sample kits that were sent out to hunters, samples from a total of 389 individuals where provided by hunters nationwide (Figure 1). Not all samples received by the lab were used in the analyses because some samples contained too few hair shafts, had hair shafts still attached to thin fragments of bloody skin, or where completely covered in blood.

**Hair cortisol analyses**

From each of the remaining 307 samples, approximately 100 mg of hair (about 20-30 hair shafts) was weighed and placed in 15 ml falcon tubes. Following the protocol developed by MacBeth et al. (2010) for grizzly bear, hair samples were washed three times in methanol (99% grade chromatography methanol). During washes, 10 ml methanol was added to the falcon tube, which was slowly rotated on a rotation device for three minutes. After three minutes, methanol was removed and new methanol added. This procedure was repeated twice for every sample. After washes, hair samples were left to air dry under a fume hood (three to five days). Samples were cut with scissors into 1-2 cm long pieces and placed in a grinding jar (25 ml stainless steel grinding jars with one 20 mm stainless steel grinding ball). Hair strands were ground into a uniform hair powder using a ball mill (Retch MM 200) at 25 Hz for 3.0 min. If hair segments were present after 3.0 min processing time, grinding was continued in 15 s intervals until a uniform powder was achieved. After grinding, 50 mg of hair powder was carefully weighed out and transferred to a 1.5 ml Eppendorf tube, into which 1 ml methanol was added. After manually shaking the tube to make sure the hair powder was evenly distributed in the methanol, cortisol was extracted by rotating the tubes slowly overnight (16 hours). The morning after, samples were centrifuged for 15 min at 4600
rev/min. 2150g, at 20 °C, and 0.6 ml of the supernatant was transferred to a glass vial, in
which it was let to evaporate in a centrifugal evaporator. Samples were reconstituted in 0.25
ml phosphate buffer and analyzed using Neogen’s commercial enzyme-linked immunoassay
kit (#402710) in duplicates. Samples were not analyzed in the order they were received by
our lab, to separate samples potentially sent from the same hunter or area over several
microplates. Regarding specificity of the ELISA, cross reactivity of the antibody used for the
cortisol kit was (according to the manufacturer): Cortisol: 100.00%, Prednisolone: 47.42%,
Cortisone: 15.77%, 11-Deoxycortisol 15.00%, Prednisone: 7.83%, Corticosterone: 4.81%,
6b-Hydroxy-cortisol: 1.37%, 17-Hydroxyprogesterone: 1.36%, Deoxycorticosterone: 0.94%,
Progesterone: 0.06%, Betamethasone: 0.05%, Dehydroepiandrosterone: 0.03%,
Dexamethasone: 0.03%, Beclomethasone: 0.01%, d-Aldosterone: 0.01%, Testosterone:
0.01%, 17α-Hydroxypregnenolone: < 0.01%, Androstenedione: < 0.01%, Cholesterol <
0.01%, Estradiol: < 0.01%, Estriol: < 0.01%, Estrone : < 0.01%, Pregnenolone: < 0.01%.
Intra- and interassay coefficients of variation (CV) were 3.9 and 15.0 %, respectively. A two-
fold increase in the amount of hair powder analyzed in a standard hair sample led to a
following two-fold increase in detected cortisol level. The detection limit was 0.4 ng
cortisol/ml (calculated by taking the average of the absorbance of the zero standard provided
with the commercial kit). This limit corresponds to a minimum detectable quantity of
approximately 0.24 pg cortisol/mg hair, which is similar to what is reported in other studies.

Variables influencing long-term stress levels

We created a priori model sets of generalized linear models by grouping parameters in
combinations that we predicted to be ecologically relevant for moose, including demographic
group, surrounding level of anthropogenic disturbance, ungulate density/competition, climate and temperature, and carnivore impact (Table 1). For the category model sets, we limited interaction terms to two-way combinations of disturbance-related variables and demographic group, as we expected risk to be perceived differently depending on sex and age. We included three variables to investigate impacts of anthropogenic disturbance; road density, human density and distance to towns. Road density and human density were quantified within a moose home range sized buffer around the kill site (1.83 km radius) in ArcMap. Road density was calculated by measuring the total road (line) length within the buffer. Distance to towns was calculated by measuring the Euclidean distance from the kill site to the nearest settlement with >200 inhabitants. Regarding temperature variables, we included four variables reflecting the conditions during the year of 2012 at the location of the shot moose (see Allen et al. (in press) and Table 1), and one climate-related variable (average temperature sum over the last 30 years, received from SMHI). The latter variable was calculated by summing the daily average temperature for days during the growing season (>5 °C), and then creating a yearly average value for 1980-2009 (according to SMHI’s product sheet). The data was delivered as average temperature sum on a sub-basin level (~500 km² on average).

Because of the similar gradients from north to south regarding human disturbance-related variables, temperature variables, and ungulate densities, it was expected that several variables would be correlated; we therefore explored collinearity for parameters using Pearson’s correlation coefficient and Variance Inflation Factors (VIFs < 2 was considered acceptable) (Zuur et al. 2010). We checked the models for normal distribution of the residuals by plotting
residuals vs. fitted values, and we compared models based on differences in Akaike's information criterion (ΔAIC). Although AIC is commonly used to select the best model from a number of candidate models, controversy exists regarding appropriate cutoffs for selecting and/or averaging top models. With recommended ΔAIC values ranging from 2 to 6 and beyond (Burnham and Anderson 2002, Richards et al. 2011), for this study, we use a cutoff value of ΔAIC of 2. We carried variables that were included in any of the category top model sets within the cutoff value forward into a comprehensive analysis of variables influencing hair cortisol levels in moose. This method enabled us to combine the most important variables and interactions from different categories for a comprehensive analysis, without the risk of over-parameterizing models and simultaneously limit the number of potentially collinear variables.

_Carnivore models_

Because there are large areas (particularly in the northern and southern parts of Sweden) where bears and/or wolves are absent, we analyzed these two variables separately for the ranges where large carnivores geographically exist. Excluding areas far from wolf territories (> 30 km from wolf territories) and where the bear index were above zero, we created two carnivore (or risk) model sets, one for bear and one for wolf, to analyze potential effects of these two species on long-term stress levels of moose. In both global models we included the effects of human disturbance, the interaction between reproductive status and carnivore occurrence, and average temperature sum (to correct for potential temperature effects). As previously, only variables kept in the top model set within ΔAIC < 2 were included in the final carnivore models. Bear index, when included as a continuous variable, generated
models with non-normal errors and marked structure in the plotted residuals, so this covariate
was divided into low, medium, and high levels (see Table 1).

*Intrinsic variables and biological validation*

We tested differences in hair cortisol levels between individuals of different reproductive
status, between individuals in poor versus healthy condition, and between groups of
individuals killed by different hunting methods. The latter two tests were included as basic
but fundamental biological validation tests of the relationship between long-term stress and
hair cortisol in moose. We hypothesized that moose in poor condition would have higher
cortisol levels than healthy individuals, and that no difference existed in cortisol levels
between moose that had been chased by hunting dogs before death compared to those shot
calmly unaware from a distance (as we measured slowly incorporated cortisol in hair). We
tested this using ANOVAs at $\alpha < 0.05$.

**Results**

Individuals in poor condition had significantly higher levels of cortisol than healthy
individuals ($F = 9.904, p = 0.002$, Figure 2). Hunting method had no effect on hair cortisol
levels of moose ($F = 0.717, p = 0.636$).

*Category variables and model sets*

Calves had substantially higher cortisol levels than adults ($F = 18.23, p < 0.001$, Figure 3),
and demographic group was carried forward into the comprehensive analysis of variables
influencing long-term stress levels in moose. Further, regarding the anthropogenic effects on
a national scale, all main effects (road density, human density and distance to towns) were
retained in the top models, as well as the interaction between road density and distance to
towns (Table 2). All ungulate species except wild boar contributed to the variation in hair cortisol levels, although only roe deer was included in all top models (Table 3).

Subsequently, to reduce the number of variables in the forthcoming comprehensive model we summed the densities for the different species to include their combined effect on moose hair cortisol levels. Regarding temperature variables, these were not included in the same model due to collinearity (Pearson’s r > 0.6 and VIFs > 2), but instead fitted to the data one by one (the best variable was determined using AIC). Average temperature sum over the last 30 years (reflecting climate) was the temperature variable explaining most of the variation in the data (AIC was 4.12 units lower than mean maximum temperature for the summer season of 2012, which was second best). Accordingly, factors and covariates carried forward into the comprehensive analysis of variables influencing long-term stress levels in moose were: demographic group, distance to towns, road density, human density, average temperature sum, ungulate density, and the interaction between distance to towns and road density.

Comprehensive model set

All variables that were included in the global model (see above) were kept in at least one of the four top models that were within ΔAIC < 2 (Table 4). The anthropogenic main effects (except road density) and ungulate density did not explain much of the variation in the data; however, demographic group, temperature sum, road density and the interaction between distance to towns and road density had significant effects (Table 5). The final model showed that calves had higher hair cortisol levels than adults and that moose in warmer regions generally had higher hair cortisol than moose in colder ranges (Table 5, Figure 4). Road density correlated positively with cortisol levels; however, in more urban areas (closer to
towns), higher road density were associated with higher cortisol levels compared to rural areas (Table 5, Figure 5).

**Carnivore models**

Higher bear index was generally negatively associated with moose hair cortisol levels (Table 6). The interaction between demographic group and bear abundance was not retained in the top models; however, an interaction plot indicated the negative effect was likely driven mainly by adults and not calves (Figure 6). Towns or roads had no apparent effect on moose within the bear range, but climate was important (Table 6). Long-term stress levels generally increased with increasing distance to wolf territories; however, females did not show this increase. On this rather small scale the effect of temperature was not important, nor were the anthropogenic effects (Table 7).

**Discussion**

Our study demonstrated that moose hair cortisol levels are not uniform across the Swedish landscape. There was a clear gradient in cortisol levels from north to south, with moose having higher levels in the south, and our analyses showed the main variable to be the distinct climatic gradient. Renecker and Hudson (1986) noted that moose decreased their activity at a temperature of 14 °C. At temperatures 20 °C moose were open-mouth panting and substantially reduced movement. For moose, the immediate responses to heat stress are increased respiration rates, decreased food intake and increased water intake (Renecker and Hudson 1986). When temperatures rise, moose increase activities that cool their bodies (e.g., wading in lakes or lying in swamps) while reducing heat-producing activities such as walking and eating. Thus, moose movement (and stress levels) could be substantially
affected by increasing temperature (Ericsson et al. 2015). Moreover, warmer temperatures increase prevalence of pathogens and parasites, which are accordingly more abundant in the southern range (Valimaki et al. 2010). Indeed, parasites were more common in envelopes containing hair samples sent to the lab from the south (data not shown). Further, moose in poor condition are more heat sensitive than healthy moose, which suggests that warming temperatures may affect health-compromised individuals more negatively than healthy individuals (McCann et al. 2013). If there is indeed a relationship between warming temperature and moose health, we may see higher moose mortality and disease rates within the next decades, especially in the southern ranges.

Our analyses indicate that, in addition to temperature effects, human activity and infrastructure affect long-term stress in moose. High road density within the home range may pose and/or be perceived as a higher risk for moose because these areas are more easily accessed by humans (Neumann et al. 2013). Our results further indicate that high road density was perceived more stressful in areas closer to towns than in rural areas, which is expected because human activity and traffic load generally is higher in urban areas. In the south where human activity and infrastructure are more prevalent, it is likely that moose become “cornered” and are less able to flee human disturbances, augmenting the effects of human activity along roads.

Interestingly, we found hair cortisol levels to be lower in connection to wolf territories and in areas with higher bear abundance, which may seem counter-intuitive but could have several causes. Areas with higher carnivore occurrence are generally characterized by having less human activity (fewer roads, towns, and human inhabitants). We did not detect the
anthropogenic variables to be important in our carnivore models; perhaps risk and human activity are working at different scales than those we have modeled here. Although we did not take different road or town sizes into account in this study, it is likely that areas with high carnivore density have fewer large roads or towns (Nellemann et al. 2007). Indeed, our data showed that areas with higher carnivore occurrence had fewer roads and were situated farther from towns (data not shown). It is possible that carnivore abundance reflect the reduced risk of human presence and/or good wildlife habitat, explaining more of the variation in the moose hair cortisol data than the anthropogenic variables included in our models. If that is the case, it could explain why roads and the interaction between roads and distance to towns were not significant variables in the carnivore models. Also, it is possible that these areas are less fragmented, which may for example mean lower ambient temperatures during hot days, as large tracts of forest exhibit a cooling effect on their surroundings (Li et al. 2015). Large carnivores have been suggested to function as umbrella species for the conservation of biodiversity (Sergio et al. 2008), and might facilitate ungulate adaptation to the ongoing climate change by constantly removing sick or otherwise weak moose individuals, which are more heat sensitive. Natural selection would thus favor individuals performing better under warmer temperatures (and potentially lower average stress levels of moose in those regions). The different pattern for moose cows in areas closer to wolf territories may be due to them perceiving risk as greater compared to males (less targeted) and calves (likely lower ability to fully assess risk).

Calves had higher hair cortisol levels than adults, which was, along with temperature, the factor that explained most of the variation in the data (occurred in all models). In ungulates,
as well as in other mammal species, younger individuals have previously been shown to have higher cortisol levels than adults (Macbeth et al. 2012, Gonzalez-de-la-Vara et al. 2011). The higher levels of hair cortisol in calves are likely due to their elevated energy metabolism during active growth, which glucocorticoids are an essential part of (Landys et al. 2006). Moose had higher stress levels in the south, and especially so in certain parts of Sweden that coincide with areas where unusually high moose mortality has been reported (data not shown). Moose in southern areas have higher presence of embryonic mortality, and high prevalence of a tick-borne pathogen that may affect moose calf health (Malmsten 2014). Therefore, the method of using hair cortisol could potentially be a promising tool to understand when and where populations are at risk of declines.

For both threatened and popular game species, declining growth rate or increased disease prevalence could lower the hunting quotas set, encourage predator control, or result in changes in land management. Such important measures should be carried out with scientific justification, and here hair cortisol analysis could potentially be an important data source. Although we found some variables to be more important than others of the ones we included in our study, the biological importance of our results, i.e., the effects on population health, is unknown. For example, at this stage we do not know what an increase in average hair cortisol level by one or two units means in terms of moose population growth. Consequently, more studies are needed before hair cortisol levels can safely be used as a management tool for wild populations.

To conclude, temperature was the most important factor that affected stress hormone levels in moose hair of the ones we included in our modeling. Earth’s average temperature is
predicted to increase by 1.4-5.8 °C the next 100 years (IPCC 2008). With many behavioral, physiological and morphological adaptations to cope with cold environments (Lundmark and Ball 2008), higher temperatures may ultimately affect moose health and distribution—which is already seen in certain areas (Lenarz et al. 2010). Further, areas without human activity may be important, as moose in such areas (which often hold large carnivores) had lower hair cortisol levels. Large carnivore populations expanding into less rural areas could potentially lead to increased allostatic load for moose.

The short term solution to lessen potential negative impacts of warming temperatures and human activity on moose and other heat-sensitive wildlife species may be to conserve continuous forests, which increase connectivity and genetic variability for populations as well as provide cooler habitats and low human activity. In particular old-growth forests greatly mitigate warmer climates with their cooling effects at local scales (Frey et al. 2016). Continuous areas with old-growth forests may thus, in addition to being hotspots of biodiversity, become increasingly important for moose and other wildlife species as global temperatures are predicted to continue to increase.

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providing temperature data. Joris P.G.M. Cromsigt was supported by a grant from the Swedish Environmental Protection Agency (NV-01337-15).
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Table 1. Variables and categories included in models to evaluate moose hair cortisol levels in Sweden 2012.

<table>
<thead>
<tr>
<th>Category</th>
<th>Variable</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intrinsic</td>
<td>Demographic group</td>
<td>Factor with three levels (female, male, calf)</td>
</tr>
<tr>
<td></td>
<td>Condition</td>
<td>Health status; factor with two levels (poor, healthy)</td>
</tr>
<tr>
<td></td>
<td>Hunting method</td>
<td>Hunting method used when killing the moose; factor with six levels</td>
</tr>
<tr>
<td>Anthropogenic influences</td>
<td>Dist. to town</td>
<td>Distance to towns (&gt; 200 inhabitants)</td>
</tr>
<tr>
<td></td>
<td>Road density</td>
<td>Road length (&gt; 5 m wide) within a 10.5 km² buffer¹</td>
</tr>
<tr>
<td></td>
<td>Human density</td>
<td>Number of humans living within a 10.5 km² buffer¹</td>
</tr>
<tr>
<td>Ungulates / competition</td>
<td>Moose</td>
<td>Number of moose shot / 1000 ha</td>
</tr>
<tr>
<td></td>
<td>Red deer</td>
<td>Number of red deer shot / 1000 ha</td>
</tr>
<tr>
<td></td>
<td>Roe deer</td>
<td>Number of roe deer shot / 1000 ha</td>
</tr>
<tr>
<td></td>
<td>Wild boar</td>
<td>Number of wild boar shot / 1000 ha</td>
</tr>
<tr>
<td></td>
<td>Fallow deer</td>
<td>Number of fallow deer shot / 1000 ha</td>
</tr>
<tr>
<td></td>
<td>Ungulate</td>
<td>Number of ungulates shot / 1000 ha (sum of all species)</td>
</tr>
<tr>
<td>Climate and temperature</td>
<td>Mean max. temp.</td>
<td>The mean of the daily maximum temperature (°C) (May 1st - August 31st, year 2012)</td>
</tr>
<tr>
<td></td>
<td>Sum above 14</td>
<td>The sum of degrees for all days where the temperature exceeded 14 °C (year 2012)²</td>
</tr>
<tr>
<td></td>
<td>Sum above 20</td>
<td>The sum of degrees for all days where the temperature exceeded 20 °C (year 2012)²</td>
</tr>
<tr>
<td></td>
<td>Days above 20</td>
<td>Number of days where the temperature exceeded 20°C (year 2012)²</td>
</tr>
<tr>
<td></td>
<td>Average temp. sum</td>
<td>Average temperature sum (years 1980-2009)</td>
</tr>
<tr>
<td>Carnivores</td>
<td>Dist. to wolf</td>
<td>Distance to wolf territories; covariate (m)</td>
</tr>
<tr>
<td></td>
<td>Bear index level</td>
<td>Bear index; factor with three levels (Low [0.00026 - 0.015], Medium [0.015 - 0.025], High [0.025 - 0.154])</td>
</tr>
</tbody>
</table>

¹Based on an average home range size with a radius of 1.83 km (southern Sweden), see Allen et al. (in press).
²Temperature based on Renecker and Hudson (1986).
Table 2. Variables included in the top generalized linear models (within ΔAIC < 2) for the anthropogenic disturbance category group to evaluate hair cortisol levels in moose sampled across the Swedish distribution in 2012.

<table>
<thead>
<tr>
<th>Model#</th>
<th>Road density</th>
<th>Human density</th>
<th>Dist. to town</th>
<th>Road density * Dist. to town</th>
<th>ΔAIC&lt;sub&gt;C&lt;/sub&gt;</th>
<th>AIC&lt;sub&gt;Cw&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td>0</td>
<td>0.28</td>
</tr>
<tr>
<td>2</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td>0.16</td>
<td>0.25</td>
</tr>
<tr>
<td>3</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td>0.82</td>
<td>0.18</td>
</tr>
<tr>
<td>4</td>
<td>+</td>
<td></td>
<td>+</td>
<td></td>
<td>0.83</td>
<td>0.18</td>
</tr>
<tr>
<td>5</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td>1.89</td>
<td>0.11</td>
</tr>
</tbody>
</table>

Table 3. Variables included in the top generalized linear models (within ΔAIC < 2) for the ungulate density category group to evaluate hair cortisol levels in moose sampled across the Swedish distribution in 2012.

<table>
<thead>
<tr>
<th>Model#</th>
<th>Roe deer</th>
<th>Moose</th>
<th>Red deer</th>
<th>Fallow</th>
<th>ΔAIC&lt;sub&gt;C&lt;/sub&gt;</th>
<th>AIC&lt;sub&gt;Cw&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td>0</td>
<td>0.23</td>
</tr>
<tr>
<td>2</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td>1.32</td>
<td>0.12</td>
</tr>
<tr>
<td>3</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td>1.37</td>
<td>0.12</td>
</tr>
<tr>
<td>4</td>
<td>+</td>
<td></td>
<td></td>
<td>+</td>
<td>1.93</td>
<td>0.09</td>
</tr>
</tbody>
</table>

Table 4. Variables included in the top generalized linear models (within ΔAIC < 2) for the comprehensive model to evaluate hair cortisol levels in moose sampled across the Swedish distribution in 2012.

<table>
<thead>
<tr>
<th>Model #</th>
<th>Average temp. sum</th>
<th>Demographic group</th>
<th>Road density</th>
<th>Human density</th>
<th>Dist. to town</th>
<th>Ungulate density</th>
<th>Road density * Dist. to town</th>
<th>ΔAIC&lt;sub&gt;C&lt;/sub&gt;</th>
<th>AIC&lt;sub&gt;Cw&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td>0</td>
<td>0.42</td>
</tr>
<tr>
<td>2</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>1.21</td>
<td>0.23</td>
</tr>
<tr>
<td>3</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>1.69</td>
<td>0.18</td>
</tr>
<tr>
<td>4</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td>+</td>
<td>+</td>
<td>1.74</td>
<td>0.18</td>
</tr>
</tbody>
</table>
Table 5. Parameter estimates (β), standard errors (se), test statistics (t-values), and p-values for variables in the final comprehensive generalized linear model to evaluate hair cortisol levels in moose across the Swedish distribution in 2012. The reference level for comparison for the categorical variable ‘Demographic group’ is ‘Bull’.

<table>
<thead>
<tr>
<th>Variable</th>
<th>β</th>
<th>se</th>
<th>t value</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Intercept)</td>
<td>-2.35e-01</td>
<td>2.56e-01</td>
<td>-0.917</td>
<td>0.360</td>
</tr>
<tr>
<td>Demographic group</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calf</td>
<td>5.11e-01</td>
<td>7.64e-02</td>
<td>6.691</td>
<td>0.000</td>
</tr>
<tr>
<td>Cow</td>
<td>-1.62e-02</td>
<td>7.00e-02</td>
<td>-0.231</td>
<td>0.818</td>
</tr>
<tr>
<td>Dist. to town</td>
<td>7.28e-06</td>
<td>8.34e-06</td>
<td>0.873</td>
<td>0.383</td>
</tr>
<tr>
<td>Road density</td>
<td>3.76e-05</td>
<td>1.43e-05</td>
<td>2.625</td>
<td>0.009</td>
</tr>
<tr>
<td>Average temp. sum</td>
<td>7.13e-04</td>
<td>2.01e-04</td>
<td>3.550</td>
<td>0.000</td>
</tr>
<tr>
<td>Ungulate density</td>
<td>3.28e-03</td>
<td>5.33e-03</td>
<td>0.616</td>
<td>0.539</td>
</tr>
<tr>
<td>Human density</td>
<td>-7.43e-05</td>
<td>8.10e-05</td>
<td>-0.918</td>
<td>0.360</td>
</tr>
<tr>
<td>Dist. to town*Road density</td>
<td>-4.68e-09</td>
<td>1.63e-09</td>
<td>-2.881</td>
<td>0.004</td>
</tr>
</tbody>
</table>

Table 6. Parameter estimates (β), standard errors (se)s, test statistics (t-values), and p-values for variables in the final generalized linear model to evaluate hair cortisol levels in moose sampled across the Swedish brown bear range in 2012. The reference levels for comparison for the categorical variables ‘Demographic group’ and ‘Bear index level’ is ‘Bull’ and ‘Low’, respectively.

<table>
<thead>
<tr>
<th>Variable</th>
<th>β</th>
<th>se</th>
<th>t value</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Intercept)</td>
<td>1.711e-01</td>
<td>2.811e-01</td>
<td>0.609</td>
<td>0.544</td>
</tr>
<tr>
<td>Demographic group</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calf</td>
<td>4.567e-01</td>
<td>1.175e-01</td>
<td>3.888</td>
<td>0.000</td>
</tr>
<tr>
<td>Cow</td>
<td>-2.261e-02</td>
<td>1.136e-01</td>
<td>-0.199</td>
<td>0.843</td>
</tr>
<tr>
<td>Average temp. sum</td>
<td>5.598e-04</td>
<td>2.381e-04</td>
<td>2.350</td>
<td>0.021</td>
</tr>
<tr>
<td>Bear index level</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medium</td>
<td>-2.329e-01</td>
<td>1.193e-01</td>
<td>-1.952</td>
<td>0.054</td>
</tr>
<tr>
<td>High</td>
<td>-1.681e-01</td>
<td>1.173e-01</td>
<td>-1.433</td>
<td>0.155</td>
</tr>
<tr>
<td>Road density</td>
<td>-1.060e-05</td>
<td>1.263e-05</td>
<td>-0.839</td>
<td>0.403</td>
</tr>
</tbody>
</table>
Table 7. Parameter estimates ($\beta$), standard errors (se), test statistics (t-values), and p-values for variables in the final generalized linear model to evaluate hair cortisol levels in moose sampled in areas < 30 km from the edge of a grey wolf territory in Sweden, 2012. The reference level for comparison for the categorical variable ‘Demographic group’ is ‘Bull’.

<table>
<thead>
<tr>
<th>Variable</th>
<th>$\beta$</th>
<th>se</th>
<th>t value</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Intercept)</td>
<td>3.221e-02</td>
<td>6.418e-01</td>
<td>0.050</td>
<td>0.9601</td>
</tr>
<tr>
<td>Demographic group</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calf</td>
<td>4.122e-01</td>
<td>2.563e-01</td>
<td>1.608</td>
<td>0.1129</td>
</tr>
<tr>
<td>Cow</td>
<td>3.103e-01</td>
<td>2.210e-01</td>
<td>1.404</td>
<td>0.1654</td>
</tr>
<tr>
<td>Average temp. sum</td>
<td>4.808e-04</td>
<td>4.484e-04</td>
<td>1.072</td>
<td>0.2877</td>
</tr>
<tr>
<td>Dist. to wolf</td>
<td>2.852e-05</td>
<td>1.301e-05</td>
<td>2.193</td>
<td>0.0321</td>
</tr>
<tr>
<td>Dist. to town</td>
<td>-9.284e-06</td>
<td>1.621e-05</td>
<td>-0.573</td>
<td>0.5688</td>
</tr>
<tr>
<td>Human_density</td>
<td>-1.362e-04</td>
<td>1.367e-04</td>
<td>-0.997</td>
<td>0.3227</td>
</tr>
<tr>
<td>Demographic group*Dist. to wolf</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calf</td>
<td>-8.294e-06</td>
<td>1.815e-05</td>
<td>-0.457</td>
<td>0.6493</td>
</tr>
<tr>
<td>Cow</td>
<td>-3.674e-05</td>
<td>1.574e-05</td>
<td>-2.334</td>
<td>0.0228</td>
</tr>
</tbody>
</table>
Figure 1. Locations of hair-sampled moose during the moose hunt, Sweden, 2012.
Figure 2. Average hair cortisol level for moose in healthy vs poor condition in Sweden, 2012. “Poor” = abnormal behavior (2), severe uterine prolapse (1), large suppuration/abscess on hind leg (1), limping/had a broken leg (3), emaciated (8). Error bars represent 95% confidence intervals.
Figure 3. Average hair cortisol levels and confidence intervals for different demographic groups of moose in Sweden, 2012.
Figure 4. Moose hair cortisol levels in relation to the long-term average temperature sum in the area where they lived.
Figure 5. Moose hair cortisol levels and road density (total road length within buffer) for grouped distances away from towns in Sweden, 2012.
Figure 6. Interaction plot showing average hair cortisol level for adults and calves in areas with low, medium, and high bear abundance in Sweden, 2012.
CHAPTER 4

The return of large carnivores amongst predator-naive prey and people

Abstract

Large carnivores have large-scale effects on their environment. When large carnivores are removed from an area, entire trophic cascades may be affected. As organisms adapt to their different, less risky environment, they may lose antipredator behaviors evolved over thousands of generations. This inexperience, or ‘naivety’, can make organisms more vulnerable to predation if predators return, and result in higher predation rates at carnivore recolonization fronts. For humans, inexperience with large carnivores may have either positive or negative consequences for large carnivore conservation depending on the context. Humans in rural areas may suffer livestock losses when carnivores return as farmers are accustomed to managing domestic animals in a predator-free environment; conversely, humans in cities may generally be positive as human-carnivore conflicts are non-existing in these areas or are benevolent in nature (e.g. bears raiding trash cans). However, there may be negative effects on large carnivore conservation when predator-naive prey and people interact in areas where apex predators recolonize, as initially high predation rates may deepen negative attitudes among stakeholders. We outline aspects of how inexperience towards recolonizing predators may affect prey, predator species, and humans, and explore potential interactions between these. We also give a few examples of how we may obtain a deeper understanding of naivety processes occurring in areas where large carnivores have been absent for decades or centuries.
**Key Words** human-wildlife conflicts, hunting, livestock depredation, large carnivores, predation, prey naivety, recolonization, Scandinavia, species interactions.

**Background**

We live in an epoch of losses. In the Anthropocene, species are disappearing at an alarming rate (Ceballos et al. 2015) and biodiversity is declining rapidly. Some of the most vulnerable species are those requiring large areas to persist, have slow reproduction, or are in conflict with human interests (McKinney 1997). Large carnivores fit all of these criteria and, as a consequence, have experienced substantial population declines and range contractions during the past two centuries (Mattson and Merrill 2002, Ripple et al. 2014).

Research over the last couple of decades indicates that large carnivores greatly impact the composition and functioning of their ecosystems (Ripple et al. 2014). Directly and indirectly they alter the behavior and density of prey species (Creel and Christianson 2008, Sih et al. 2010), suppress mesopredator populations (Elmhagen and Rushton 2007), affect vegetation (Ripple and Beschta 2007), and affect the behavior and traditions of humans (Gangaas et al. 2013). The extensive and complex impacts of large carnivores are thus far-reaching across trophic levels and ecosystems.

Notably, humans have the power to dramatically and purposefully alter numerous features of the environment they live in. Thus, humans now join large carnivores at the apex position of ecosystems, and affect several stages of the food web (Haswell et al. 2016). Many of the same features that put large carnivores at the apex of trophic systems also make them vulnerable to human exploitation and persecution, which have consequences for their ecological functionality and evolution (Ordiz et al. 2013). It is thus not surprising that
massive conflicts occur between humans and large carnivores, as the two compete at the top of the food chain for both space and sustenance (Chapron and Lopez-Bao 2016). These conflicts generate negative attitudes towards large carnivores in many areas across their range (Gangaas et al. 2013), which in turn is deemed as one of the greatest threats against their successful conservation (Ripple et al. 2014).

Large carnivores are now recolonizing some of the regions where they have been absent for an extended time (Chapron et al. 2014) (Figure 1); however, the historical eradictions of these species may have had long-lasting impacts on prey and humans. Prey species may have lost a proper antipredator behavior—a crucial component of their continuing survival in a world with returning predators (Sih et al. 2010). Humans have changed their way of life as large carnivores mainly became stories from the past (Gangaas et al. 2013). In the next sections we review and explore the adjustments that prey and people make when apex predators become functionally extinct across wide ranges, with implications for large carnivore recovery.

**There and back again: repercussions of a carnivore comeback**

*Predator-naive prey*

When predators are present prey species have little choice—they need to hide, move away, or defend themselves to survive (Lima and Dill 1990). Prey species have evolved antipredator traits that enable them to reduce predation risk throughout the landscape (camouflage, aposematism, group living, vigilance [wariness], and armaments [e.g., antlers or venom] to mention a few). However, antipredator responses come with costs: although the responses increase the chance for an individual to survive in a dangerous landscape, they may impact
negatively on reproduction and population growth (Creel et al. 2007). As a result, prey species minimize their antipredator behavioral responses so that more time can safely be spent on other important activities, such as foraging or resting (Lima and Dill 1990). Thus, in the extreme situation, when carnivores go locally extinct, prey should lose costly antipredator behaviors over time.

The loss of expressed antipredator responses can generally occur on ontogenetic (based on lifetime experience and training) or evolutionary time scales (Griffin et al. 2000). An individual that experiences reduced predation risk over the course of its lifetime may decrease time spent being vigilant, as these behaviors simply are not needed in a world without predators. However, should carnivores return within that individual’s lifetime, the required vigilance would most likely quickly be re-established, as the behavior is kept in memory (as memories per se are not particularly costly). Across multiple generations, however, there is a chance that costly antipredator adaptations are lost as there is a fitness component involved (Griffin et al. 2000, Sih et al. 2010). More explicitly, if large carnivores have disappeared, individuals without inherent antipredator behaviors may be equally or more likely to survive as individuals with antipredator behaviors. Gradually, unless antipredator responses have become fixed in the population so that natural selection cannot operate, the frequency of individuals without inherent antipredator behavior may increase over generations. As large carnivores disappear, there is a chance that certain behavioral syndromes (sets of behavioral traits) in the prey species disappear with them, creating so-called ‘naive’ prey (Sih et al. 2010). These losses would potentially go unnoticed unless predators are let to recover in the future.
To date, the variation in prey retention of antipredator behavior is largely unknown but is of great conservation value. Some studies show that antipredator behavior may be still be present after hundreds or even thousands of years after predator extinction (Blumstein and Daniel 2002, Blumstein 2006), whereas others indicate that prey lose antipredator behaviors within a few generations (Berger et al. 2001, Berger 2007) but may retain the ability to regain antipredator behavior relatively fast (Berger et al. 2001).

Importantly, effective antipredator behaviors reduce predation, but result in non-consumptive effects (Schmitz et al. 1997, Preisser et al. 2005). As shown by Preisser et al. (2005), such indirect or non-consumptive effects from predators may be larger than the direct effects when it comes to limiting prey population size (in other words; the actual killing of prey individuals is limiting prey populations less than the energetic cost of their antipredator behaviors). In an extreme scenario, predator effects in areas with naive prey are exclusively of the direct (consumptive) nature, in contrast to predator effects in areas where they have coexisted over time where both direct and indirect effects occur (Sih et al. 2010).

Thus, the first phase of large carnivore recovery may exert a strong selection on the prey species by removing naive individuals, which explains why naivety may fade relatively quickly in prey species once predators are established. Nonetheless, any negative impacts of naivety are important to consider before carnivores return to specific regions, as they may affect prey populations strongly, and human attitudes towards large carnivores. A few examples on prey naivety and their implications are given in Table 1.
Predator-inexperienced people

Similar to prey species, humans historically evolved behaviors and traditions to cope with the presence of large carnivores, not only because of risks of carnivore-induced injury or death for the humans, but, perhaps even more importantly because of costly impacts of carnivores on human practices (e.g., animal husbandry). Shepherding, livestock-guarding dogs, and nighttime enclosures are all examples of antipredator activities that humans evolved. However, again similar to prey, these antipredator behaviors involve indirect costs. For example, preventing large carnivore-human conflicts comes with material and economic costs involved with fencing, training of guard dogs, etc. Consequently, when carnivores disappear from a system, animal husbandry and hunting traditions are gradually adjusted to become more efficient in the less risky environment. Thus, protective systems are not needed for safety of the livestock, and big game hunting traditions change, partially because hunting dogs can be used more extensively (primarily in the Scandinavian countries).

Further, as changes in traditions happens over relatively long time, people’s perceptions change or drift longer away from the true natural conditions (Pauly 1995). Such baseline shifts may result in a greater resistance for people to tolerate large carnivores and abide the regulations to conserve them when large carnivores are recolonizing. Although there are conflicts between large carnivores and people in areas where they have coexisted continuously, conflicts with large carnivores are often more severe when these animals return to areas from which they have been absent for decades or centuries (Gangaas et al. 2013). For example, people inhabiting areas where carnivores are reintroduced tend to be less tolerant of carnivores compared to people in areas where carnivores have continuously existed for a
long time (Roskaft et al. 2003, Teel and Manfredo 2010). Thus, in the absence of carnivores, humans, similar to predator-naive prey species, may forget how to coexist with these large animals. And, once carnivores are gone, why go “back” to the demanding life with roaming large carnivores and their associated risks when it is more convenient without them? This question implies that some humans may not want to change to a lifestyle adapted to cope with carnivores, because of costs associated with it, or because of strong cultural norms.

For example, in addition to conflicts between farmers and large carnivores, certain groups of hunters may be negatively impacted by large carnivore recovery. Hunters may see large carnivores as competitors and are not willing to share game species with another species (Ericsson et al. 2004, Gangaas et al. 2013). Another reason is that popular hunting methods have not been properly adapted to the elevated risks when large carnivores are present. For example, the most common factor involved when humans have been injured by bears *Ursus arctos* in Scandinavia is moose *Alces alces* hunting using baying dogs (Sahlén 2013). The dog starts following a bear, which chases the dog back to the owner—an owner often unaware of the dog returning with a bear in hot pursuit. Within wolf *Canis lupus* territories, baying dogs may be injured or killed (Karlsson 2001) as wolves naturally defend their territories against trespassing canines. Thus, certain hunting traditions involve a great deal of risk taking in areas where large carnivores are returning, as they may cause great danger for hunters and dogs. However, hunting with dogs has become culturally and socially important (Heberlein 2000), especially in rural areas, and is an extremely sensitive issue around the borders of wolf territories. When dog fatalities occur it augments negative attitudes against
large carnivores even further, not the least due to the massive attention large carnivore attacks gets in local and national media (Bisi et al. 2007).

Wikenros et al. (2015) showed that hunters are less likely to harvest as many individuals in areas where large carnivores returned. One reason is hunters’ intentions to give room for natural predation without exhausting the prey base (Wikenros et al. 2015); however, such intentions may not be optimal as recommended hunting quotas may not be met. Moreover, the actions of hunters in areas at large carnivore recolonizing fronts may indirectly impact on lower trophic levels, as hunters kill less big game (Wikenros et al. 2015) and thereby initiate an increase in ungulate browsing damage in certain forest stands. To our knowledge, these effects do not have a scientific basis; however, it is evidently a perception that exists within certain regions.

Interestingly, human inexperience with large carnivores may have either positive or negative consequences for large carnivore conservation depending on the context. Inhabitants in rural areas generally have a more negative attitude towards carnivore restoration, whereas people living in more urban areas are more accepting (Ericsson and Heberlein 2003). People living in cities have never experienced the potential negative impacts of having large carnivores ‘in their backyard’, and are generally positive about these animals’ recovery. Nevertheless, the debate about large carnivores has to some extent been about if carnivores are dangerous to humans or not (Roskaft et al. 2003, Penteriani et al. 2016). Fear of large carnivores is widespread and even more common in areas with low large carnivore densities (Ericsson and Heberlein 2003, Bisi et al. 2007) and people may be reluctant to go berry or mushroom picking in areas where recent wolf or bear sightings have been reported. Clearly,
information and ways to deal with these multiple and complex issues above must be provided to reduce the negative impacts of recolonizing large carnivores on people and their way of life.

*Predator-naive prey and people may interact*

In areas where prey species have become naive to predators and lost their antipredator responses, the initial predation rate may be several times higher compared to areas where prey and predators have coexisted for centuries (Martin 2014). If predator-naive prey and people overlap in their distributions, there may be a negative interaction of these on large carnivore conservation. Because, not only are carnivores returning and traditions forced to change, carnivores are also removing more game than what was expected (or stated) before their return. Importantly, unexpected outcomes will be reduced if managers have access to more accurate information about expected predation rates in certain areas when large carnivores recolonize, as predator-inexperienced people could become better prepared. As large carnivores often are seen as competitors for big game hunters (Ericsson et al. 2004), and the hunter lobby is extremely strong in certain areas, such knowledge may help to reduce negative attitudes. Essentially, much of the negativity about large carnivore recolonizations could be minimized if more focus of wildlife management in affected areas was planned from a ‘naivety’ perspective, for prey and people.

Furthermore, human activities may be one of the drivers of naivety in prey species in human-modified landscapes. For example, hunting with loose baying dogs for decades could potentially affect how ungulate respond to wolves when these return (Sand et al. 2006, Ericsson et al. 2015). Hunting is only one example and other human disturbances may work
in similar ways. Thus, human activities may be an important factor determining whether prey lose or maintain antipredator responses over time.

**Synthesis and implications for conservation**

Conserving large carnivores is important for several reasons and the need for action is immediate (Ripple et al. 2014), especially where carnivores are now reestablishing. Similar to prey species, negative direct effects of carnivores are more widespread for humans if antipredator measures commonly used in the past are missing. Thus, in areas where humans still use the more traditional protective systems with shepherds, livestock-guarding dogs, and night-time enclosures, the numbers of livestock killed are smaller, indirect costs higher, and human-wildlife conflicts fewer. However, when the protective systems have been reduced, with livestock grazing unattended in forests or mountain pastures, direct losses may be more severe and conflicts substantial. It is under these circumstances that large carnivore recovery often is particularly challenging. Thus, there is a constant tradeoff between indirect and direct costs of predation for humans and prey species; however, with neither experience nor training, indirect effects will be nearly absent, and direct effects will be strong.

When carnivores return, the few studies conducted indicate that naivety appears to fade within a few generations in prey species (e.g., Berger et al. 2001), but knowledge about factors affecting human resistance to change with respect to large carnivore conservation, and how this resistance fades over time, is still limited. It has been proposed that the younger, more educated generation that move back to rural areas from big cities after completing higher education may represent a potential contrast and dilute the old traditions and farming values in certain areas (Gangaas et al. 2013). If ensuing human generations in areas where
large carnivores are returning will be more accepting than were their descendants, the future for large carnivore conservation is certainly looking brighter. Below we highlight three focus areas for future research that will help managers predict and ameliorate the outcomes of recolonizing or invading predators in terms of the effect on humans and prey species.

Naivety assessment

Under what circumstances do prey become predator-naive, and what determines the speed of antipredator behavior loss and gain? Both new studies and meta-analyses of prey naivety studies could be used to assess generalities in drivers of naivety loss and the speed of this loss. Such meta-analyses would also identify knowledge gaps. Naive prey species could potentially receive antipredator training, which is often considered in certain areas but rarely implemented (McLean et al. 1999). Potentially, there are methods to better prepare predator-inexperienced humans in focal areas, which would consist of informing affected people about potential impacts such as predation rates on livestock, costs of fencing, potentially decreasing hunting quotas, and general information about large carnivore ecology, to mention a few. Surveys and information about how large carnivores will affect them and their practices are pivotal for the outcome of large carnivore recovery in these areas.

Naivety and relative importance of direct and indirect effects

More use should be made of natural experiments created by carnivore recolonization events. There is great potential to start measuring prey and human behavior in areas where large carnivores have continuously been present versus areas that they are recolonizing. By quantifying the relative importance of indirect versus direct effects along these gradients, we can get better insights in the drivers of variation in prey naivety and human perceptions. This
is important, because the extent to which antipredator responses have been maintained over time by prey (and humans), determines the impact carnivores will have on prey and human societies. Greater knowledge about these processes would therefore help minimize large carnivore-human conflicts and negative attitudes in predator-inexperienced humans.

Spatial modeling

Studies have used spatial modeling to predict where human-large carnivore conflicts are likely to occur depending on e.g., human attitudes, the abundance of farmland, or other landscape features (Roskaft et al. 2007, Majic and Bath 2010, Gangaas et al. 2013, Behdarvand et al. 2014). Similar studies can be carried out to predict where prey species and people are the most likely to be negatively impacted by large carnivore recovery, by creating overlapping intensity maps over predator-naive prey and predator-inexperience in people. These models should be based on various important aspects in addition to the ones used previously, and include aspects of hunting traditions in the area, game species importance for hunters, livestock depredation, time of large carnivores absence, etc. These integrative measures can be used to predict where intensive management and conservation efforts should be focused proactively and in what way, even before large carnivores recolonize an area.

Concluding

Assessing the level and nature of naivety, or inexperience, in prey and people with respect to large carnivores is important. This will hopefully i) give us more information about how to ameliorate naivety processes, ii) generate knowledge to help reduce negative attitudes towards large carnivores and hopefully decrease poaching rates, iii) reduce the probability of negative effects on mending carnivore populations in areas where both prey and people are
predator-inexperienced, and where predators and people compete for big game, and iv)
provide information for decision-making processes regarding expensive large carnivore
reintroduction programs, as these programs will remain unsuccessful in areas where people
are not willing or able to adapt to change. We argue that tackling these issues is imperative
for the conservation of large carnivores worldwide, as the fate of their persistence lies in
people’s willingness to coexist with them.
REFERENCES


Haswell, PM. et al., 2016. Large carnivore impacts are context-dependent. (In Press, Corrected Proof) http://dx.doi.org/10.1016/j.fooweb.2016.02.005


Table 1. Examples of processes and situations related to carnivore eradication and recovery with implications for conservation.

<table>
<thead>
<tr>
<th>Study</th>
<th>Species and location</th>
<th>Process Description</th>
<th>Description</th>
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<tbody>
<tr>
<td>Frair et al. 2007</td>
<td>Elk <em>Cervus elaphus</em> – Alberta (Canada)</td>
<td>Increased direct effects in terms of lower survival of introduced predator-naive prey</td>
<td>Elk with different background experiences were experimentally released into an area holding predators, hunters, and a varying habitat quality. During the first year, familiarity with either wolves or hunters prior to release yielded survival rates 1.9-2.2 times greater than what was observed for animals naive to both risks. Second-year survival rates were equivalent to the ones for resident elk.</td>
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<td>Blumstein et al. 2004</td>
<td>Tammar wallaby <em>Macropus eugenii</em> – Australia and New Zealand</td>
<td>Lost antipredator behavior</td>
<td>To study predator recognition, authors observed the response of New Zealand wallabies to the presentation of taxidermic mounts of mammalian predators, and to broadcasted sounds of mammalian and avian predators. In contrast to control groups, complete isolation from all predators for as few as 130 years led to the loss of group size effects and a rapid breakdown in visual predator recognition abilities.</td>
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<td>Berger 1999</td>
<td>Moose <em>Alces alces</em> – Alaska and Rocky Mountains (USA)</td>
<td>Lost interspecific interactions between species, lost ability to respond to environmental predator cues</td>
<td>In Alaska (where scavengers and large carnivores are associated with carcasses), field experiments demonstrated that moose is sensitive to the vocalizations of ravens <em>Corvus corax</em> and may rely on their cues to avoid predation. However, a similar relationship is absent where grizzly bears and wolves have been extinct for 50-70 years.</td>
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<tr>
<td>Bisi et al. 2007</td>
<td>Humans – Finland</td>
<td>Strengthened negative attitudes towards large carnivores, lost general knowledge about large carnivore behavior, altered way of life</td>
<td>The study was based on the preparation process of the wolf management plan in Finland, where a nationwide hearing was arranged in 2004. In areas where the wolf population was low or no wolves existed, people were the most negative and wanted wolf-free areas, whereas in the regions with a stable population such opinions were rare. Hunters and municipality representatives were most eager to decrease the wolf population, and their explanations were related to the damage and fear wolves inflicted. Moreover, issues classified as “fear and concern” and “problems with dog keeping and hunting” were more common in the areas where the wolf population was low or where no wolves existed at all.</td>
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1Group size effects - the way in which animals change the time they allocate to antipredator vigilance as a function of group size.
Figure 1. Current distribution of brown bear (top) and grey wolf (bottom), and their lowest distributions after the end of eradication campaigns in the 20th century. Human tolerance of large carnivores tends to be higher in areas where carnivores have existed for a long time (dark green) compared to areas they now recolonize. Map is based on data from Chapron et al. (2014).