Avian predation on a parasitic fly of cervids during winter: can host-related cues increase the predation risk?

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The deer ked (Lipoptena cervi) is an ectoparasitic fly on cervids that has expanded its distribution rapidly in Northern Europe. However, the regulating biotic factors such as predation remain unknown. The host-independent pupal stage of the fly lasts for several months. Blackish pupae are visible against snow, especially on the bedding sites of hosts, and are thus exposed to predators. To evaluate the role of predation on the invasion dynamics and evolution of L. cervi, we monitored pupal predation on artificial bedding sites in three geographical areas in Finland during winter. We explored: (1) possible predators; (2) magnitude of predation; and (3) whether predation risk is affected by host-derived cues. We demonstrate that pupae are predated by a number of tit species. Any reddish brown snow discoloration on bedding sites, indicating heavy infestation of the host, serves as an exploitable cue for avian predators, thereby increasing the risk of pupal predation. The ability of tits to use this host-derived cue seems to be dependent on the prevalence of L. cervi and the period of invasion history, which suggests that it may be a learned behavioural response. Predation by tits may potentially affect the L. cervi population dynamics locally. © 2012 The Linnean Society of London, Biological Journal of the Linnean Society, 2012, 106, 275–286.


INTRODUCTION

The successful establishment of invasive species in new environments depends on both abiotic and biotic factors, with the latter group including interactions with the resident biota (Vermeij, 1996; Lockwood, Hoopes & Marchetti, 2007; Menke et al., 2007). Predation is one of the major biological factors that regulates prey populations, and in some cases can affect their invasion success (e.g. Schoener & Spiller, 1995; Kotiaho & Sulkava, 2007). Natural enemies may show a preference for the invader and thereby even prevent its establishment (e.g. Barber, Marquis & Tori, 2008; Carlsson, Sarnelle & Strayer, 2009). On the other hand, the enemy release hypothesis argues that invasion success of a new arrival results from reduced natural enemy attack (e.g. Williamson, 1996; Shwartz et al., 2009).

Predation on the pupal stage of insects has been reported, especially by small mammals, insectivorous birds, and arthropods (e.g. Frank, 1967; Tanhuanpää et al., 1999; Hastings et al., 2002; Barbaro & Battisti, 2011). In the northern boreal region, endothermic vertebrates are potential predators of insects during
the winter (e.g. Alatalo, 1980; Jansson & von Brömssen, 1981). Birds and small mammals may use olfactory, auditory, and visual cues when searching for prey (Bennett & Cuthill, 1994; Montgomerie & Weatherhead, 1997; Nevitt, Losekoot & Weimerskirch, 2008; Vaughan, Ryan & Czaplewski, 2011). Olfactory cues such as volatile organic compound (VOC) emissions by damaged plants may enhance the prey location efficiency of insectivorous birds (Mäntylä et al., 2008). According to current understanding, however, the predominant mode of prey detection among birds is vision (see Rajchard, 2009 for a review). Many birds can detect ultraviolet light and/or chromatic cues when searching for prey (Bennett & Cuthill, 1994; Viitala et al., 1995; Church et al., 1998; Rajchard, 2009). Moreover, indicators like leaf damage (e.g. Heinrich & Collins, 1983) or the specific feeding habits (Murakami, 1999) of leaf-feeding caterpillars can serve as visual cues for insectivorous birds. Although predation by birds may potentially regulate invertebrate populations, in general with the highest prey mortality at low prey density (Fowler et al., 1991), the effects on insect population dynamics are not well understood. Even less is known about predation as a potential regulator of the off-host stages of ectoparasites.

The deer ked, Lipoptena cervi (Diptera; Hippoboscidae), is an obligate haematophagous ectoparasite of several species of cervids (Haarlov, 1964). It can affect the health of its host (Kynkäänniemi et al., 2010) and is a nuisance to humans: for example, causing allergic reactions (Rantanen et al., 1982; Kortet et al., 2010). In Northern Europe, L. cervi has rapidly expanded its distribution and increased in abundance during the preceding four decades (Välimäki et al., 2010; Kaunisto et al., 2011). Yet, there is significant spatial variation in the expansion rate of the species (Välimäki et al., 2010). To date the role of potentially limiting biotic factors such as predation has not been studied, even though Haarlov (1964) proposed the possibility almost 50 years ago. To understand the ecology and evolutionary invasion dynamics of L. cervi, the interactions between the parasite, its hosts and possible predators need to be studied.

We examined pupal predation as a possible factor affecting the population dynamics of L. cervi. The pupal stage is independent of the mobile warm-blooded host, and may therefore be among the most vulnerable stages to extrinsic mortality agents. First, we explored whether there is predation on L. cervi pupae in the Northern Boreal region during winter. We assumed that tits (Paridae), which are highly adaptable predators (Pimentel & Nilsson, 2007) and form feeding flocks during winter (Suhtonen, 1993; Suhonen, Alatalo & Gustafsson, 1994), would be the most likely predators. Second, we examined the effects of host-related factors upon the probability of predation. In general, factors like body fluids and secretions from the prey may increase the predation risk, especially by avian predators, because of the UV visibility of secretions (e.g. Viitala et al., 1995; Koivula & Korpimäki, 2001). The snow covering bedding sites of infested cervids is characterized by the reddish brown discoloration of tissue fluid and blood from the host (caused by L. cervi bites and irritation of skin) and L. cervi faeces, with the degree of discoloration correlating positively with infestation intensity (Kaunisto et al., 2009; Välimäki et al., 2011). Hence we hypothesized that parasite-induced snow discoloration on host bedding sites may serve as a cue of the presence of parasites, and thus increase the predation risk. Associated with the contour of a bedding site, host faecal pellets may provide an additional cue by revealing the previous presence of the host, and thereby indicating a potential foraging site. This is a reasonably assumption, as cervids often feceate onto their bedding site once they leave it, and brownish faecal pellets are more conspicuous against snow than clean bedding sites as such. By using different combinations of cues, we were able to disentangle whether the host-derived cue (faecal pellets) expose pupae to predation or whether the infestation-induced cue (snow discoloration) triggers the behavioural response in possible predators. Moreover, we evaluated whether the ability to exploit certain cues is innate in predators or a learned response to reliable cues. This knowledge would also allow us to better understand evolutionary mechanisms of the host–parasite interactions in the present study system.

MATERIAL AND METHODS

NATURAL HISTORY OF LIPOPTENA CERVI

Lipoptena cervi is a Palearctic species that has been introduced to the Nearctic region (e.g. Maa, 1969; Dehio, Sauder & Hiestand, 2004). In Fennoscandia, especially in Finland, L. cervi has rapidly spread north up to the southernmost part of the region of reindeer husbandry (Hackman, Rantanen & Vuojolahti, 1983; Välimäki et al., 2010). In Finland, the main breeding host (i.e. host supporting reproduction) of L. cervi is the moose (Alces alces) (Välimäki et al., 2011). A single A. alces bull may host up to 17 500 adult L. cervi (Paakkonen et al., 2010). The wild forest reindeer (Rangifer tarandus fennicus) (Kaunisto et al., 2009) and occasionally the semi-domesticated reindeer (Rangifer tarandus tarandus) (Kynkäänniemi et al., 2010) can also serve as hosts. Lipoptena cervi can also parasitize the roe deer (Capreolus capreolus), the fallow deer (Dama dama), the red deer (Cervus elaphus) (Haarlov, 1964), and the
white-tailed deer (*Odocoileus virginianus*) (Matsunoto et al., 2008), but this does not seem to be the case in Finland (Välimäki et al., 2011). The increased density of *A. alces* is likely to be the main reason that underlies the rapid spread and increase of the ectoparasite in Finland (Välimäki et al., 2010). In addition to the host availability, ongoing climate warming may be a factor that enhances the range expansion (Härkönen et al., 2010).

Adult *L. cervi* emerge during late summer until the onset of winter (Hackman et al., 1983). Immediately after finding a host, flies shed their wings and start to feed on blood (Bequaert, 1953). The adults spend wintertime on the same host, with viviparous females giving birth to already prepupated larvae. Offspring are produced one at a time, with the female’s reproductive output being dispersed evenly across the whole reproductive phase, which lasts from autumn to the following spring (Haarløv, 1964; Popov, 1965). The boreal areas are largely covered by snow at that time of year. Pupae fall off the host and are thus exposed to extrinsic mortality factors for several months until the emergence of the new adult generation in the following autumn.

Blackish and oval-shaped (c. 3 mm in diameter) pupae fall off the host during host activity and rest. The resting bouts of *A. alces* may last for more than 18 h a day in the winter (see Van Ballenberghe & Miquelle, 1990). Assuming a constant reproductive rate of female *L. cervi*, about three-quarters of their reproductive output would thereby be exposed to predation on host bedding sites. Depending on the infestation intensity, the total number of pupae produced by a group of females that reproduce simultaneously on a particular host individual varies from zero to dozens on natural bedding sites (Välimäki et al., 2011). We have observed only a few dead adult *L. cervi* on *A. alces* bedding sites during the winter months, which together with a constant reproductive output suggests relatively low on-host mortality of reproductive adults (P. Välimäki, pers. observ.). Thus, the pupa stage is likely to be the most vulnerable developmental stage, and on the other hand the bedding sites are locations where predation may be intense enough to affect *L. cervi* population dynamics.

**STUDY AREAS AND EXPERIMENTAL SET-UP**

We conducted the study in five localities, representing three geographical areas (Fig. 1). The study took place during late winter, in March and early April 2009. Konnevesi (62°41′N, E 26°16′E) is situated in southern Central Finland, whereas the other localities are situated in northern Central Finland. Of the latter areas, Rantsila (64°28′N, 25°48′E) and Pulkkila (64°18′N, 25°47′E) fall within the current core area of *L. cervi*, whereas Utajärvi (64°45′N, 26°53′E) and Yli-Il (65°26′N, 26°10′E) are closer to its recent expansion front (Fig. 1). We chose the study sites based on *A. alces* and *L. cervi* densities (see below). Also, the occurrence of the assumed predator guild, the tits, affected the study site selection. A rough estimation of tit species occurrence on each study site was based on visual and auditory observations on tit species made before the onset of the experiments.

We produced artificial bedding sites by digging grooves [150 cm × 75 cm × 30 cm (depth)] onto snow to imitate natural *A. alces* bedding sites. We provided the artificial bedding sites with *L. cervi* pupae as well as potential host-derived cues, such as *A. alces* faecal pellets and snow discoloration (discoloration directly indicates *L. cervi* infestation), and monitored the pupal loss caused by predators. We manipulated the artificial bedding sites in increasing order of visual and olfactory cues, revealing the feeding site for potential predators. The number of experimental manipulations varied among the study questions I–III (Table 1). We placed *L. cervi* pupae near an imaginary neck area of the artificial bedding site to mimic natural infestation (Kaunisto et al., 2009). Because the snow melts underneath a resting large-bodied endotherm, the upper snow layer of natural bedding sites becomes very dense, which prevents pupae from sinking into the snow, leaving them visible for relatively long times. To prevent the pupae from sinking and to estimate the maximal predation risk, we placed the pupae on a white piece of gauze (20 cm × 20 cm). We removed fresh snow in the case of snowfall. When applicable, the number of *A. alces* faecal pellets was ~20. In two experimental manipulations, we created snow discoloration by spraying a water solution that consisted of reindeer blood and yellow and brown watercolour pellets onto the imaginary neck and back region of the artificial bedding sites. This was supposed to mimic the natural snow discoloration on bedding sites (Kaunisto et al., 2009; Välimäki et al., 2011). The artificial discoloration was composed mainly of reindeer blood, which ensured a close resemblance to the natural discoloration not only visually but also by other properties, such as UV-reflectance, which appears to be important for foraging birds (Bennett & Cuthill, 1994; Church et al., 1998; Koivula & Korpimäki, 2001). We adjusted the magnitude of artificial discoloration to correspond to the natural bedding site of a highly infested host.

Depending on the experimental set-up, distances between individual bedding sites or groups of bedding sites (replicates) were ~0.5 km, so as to avoid pseudoreplication. Each replicate was located within predominantly coniferous forests. We monitored the artificial bedding sites at 8, 24, 48, 72 and 116 h after the onset of experiments. At each visit to the artificial
bedding sites, which lasted for c. 10 min, we counted the number of pupae and recorded all auditory and visual observations of potential avian predators within ~50 m radius from a particular replicate. We also recorded signs such as subnivean tunnels, tracks, and faeces of possible small mammalian predators like voles, shrews, and mice in the immediate vicinity of each artificial bedding site (within ~5 m radius). Of the avian species, we concentrated on tits, which are the known wintertime predators in the Northern Boreal forests (see Alatalo, 1982; Suhonen, 1993; Pimentel & Nilsson, 2007). During typically cold and short winter days, tits, especially the willow tit (*Poecile montanus*) and the coal tit (*Periparus ater*) are very sedentary as long as food accessibility remains sustainable (e.g. Brotons, 1997; Lahti *et al.*, 2007).

**Figure 1.** Locations of the study sites in Finland (black dots). The current geographic range of *Lipoptena cervi* (grey line) is defined according to Valimäki *et al.* (2010).
Table 1. Total number of impacted bedding sites and the percentage of eaten pupae (in parenthesis), in relation to the study questions and experimental manipulations of bedding sites in each locality, with varying Lipoptena cervi abundance. The study questions: I, are pupae predated upon at bedding sites?; II, do host-related cues expose pupae to predation?; III, does natural variation in L. cervi infestation intensity affect predation risk?

<table>
<thead>
<tr>
<th>Location</th>
<th>L. cervi abundance</th>
<th>Study question</th>
<th>Experimental manipulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Konnevesi (62°41′N)</td>
<td>high</td>
<td>I</td>
<td>Pupae</td>
</tr>
<tr>
<td>Pulkkila (64°18′N)</td>
<td>high</td>
<td>I</td>
<td>Pupae</td>
</tr>
<tr>
<td>Rantsila (64°28′N)</td>
<td>high</td>
<td>II, III</td>
<td>Pupae + faeces</td>
</tr>
<tr>
<td>Pulkkila (64°18′N)</td>
<td>high</td>
<td>III</td>
<td>Pupae + colour</td>
</tr>
<tr>
<td>Utajärvi (64°45′N)</td>
<td>low</td>
<td>III</td>
<td>Pupae + faeces + colour</td>
</tr>
<tr>
<td>Yli-Ii (65°26′N)</td>
<td>low</td>
<td>III</td>
<td>Pupae</td>
</tr>
</tbody>
</table>

1998). Thus, the distance of 0.5 km decreased the possibility that the same tit individuals would visit several replicates during the experiment. We admit that our experimental design is not totally comprehensive because, for example, coal tits may shift their foraging habitat preference in winter, and increase their range in search of new food resources (see, e.g., Brotons & Herrando, 2003). However, we stress that the transects of replicate experimental units were from 8 to 10 km in length, and thus the assumption of independence was probably not severely violated.

Are pupae predated on bedding sites?
First, we studied if any pupal predation occurs in the absence of other host-related cues, except for the contour of an artificial bedding site. We conducted this experiment in Konnevesi and Pulkkila, which are characterized by abundant A. alces and L. cervi populations (see Välimäki et al., 2010, 2011), with the respective number of replicates (artificial bedding sites) being 16 and 20 (see Appendix S1). The number of pupae on each bedding site was 15.

Do host-related cues expose pupae to predation?
The aim of this experiment was to study whether predators can take advantage of cues that derive either from the cervids themselves (faecal pellets) or from the interactions between the host and the parasite (snow discoloration), and if the response of the predator is cumulative to these cues. We performed this experiment in Rantsila, which had an abundant and heavily infested winter population of A. alces at that time [proportion of infested A. alces, 0.96 (N = 46); average number of pupae on natural bedding sites, 7.46 ± 1.58 (95% CI); data from Välimäki et al., 2011]. We performed four different experimental manipulations using artificial bedding sites with: (1) just pupae; (2) pupae and A. alces faecal pellets; (3) pupae and snow discoloration; or (4) pupae, snow discoloration, and A. alces faecal pellets (see Appendix S1). We organized the artificial bedding sites into a 40-m × 40-m quadrat, with each corner standing for one of the four manipulations. We replicated the set-up 20 times so that the exact locations of artificial bedding sites (i.e. particular manipulations) were randomized within each quadrat. Hence, we constructed a total of 80 artificial bedding sites. The number of pupae on each bedding site was 10.

Does natural variation in Lipoptena cervi infestation intensity affect predation risk?
Finally, we constructed additional experimental set-ups to explore whether the patterns observed in Rantsila can be generalized, or whether there are spatial differences in predatory response to the host-derived cues (see Appendix S1). We organized 16 pairs of artificial bedding sites within each locality so that the exact locations of two experimental manipulations (pupae only and pupae with snow discoloration) were randomized within each pair. We applied only two of the four possible manipulations because snow discoloration appeared to be the most important cue for predators in the former trial (see Results). The number of pupae on each bedding site was 10.

The four study localities (Rantsila, Pulkkila, Utajärvi, and Yli-Ii) varied in relation to A. alces and L. cervi abundance, but the main avian predators, tits, were observed near the artificial bedding sites in each locality. In Utajärvi, the A. alces winter population was quite similar in abundance to the one in Rantsila, but L. cervi was less abundant in the former locality [proportion of infested A. alces, 0.81 (N = 42) versus 0.96 (N = 46); average number of pupae on natural bedding sites, 1.86 ± 1.13 (95% CI) versus 7.46 ± 1.58 (95% CI); data from Välimäki et al., 2011]. In Yli-Ii, the A. alces winter population was scarce,
and as an indication of that, we did not detect any A. alces tracks during the experiment in the area. The abundance of L. cervi was also very low, as neither the seven natural bedding sites found ~5 km east of the study site nor the 12 additional bedding sites inspected in 2010 and 2011 within the study site showed any signs of L. cervi infestation (only one pupa was found on a natural bedding site of a semi-domesticated reindeer; N = 40). On the contrary, the study area of Pulkkila was very similar to Rantsila, both in terms of A. alces and L. cervi abundance [proportion of infested A. alces, 0.90 (N = 10) versus 0.96 (N = 46); average number of pupae on natural bedding sites, 5.60 ± 2.65 (95% CI) versus 7.46 ± 1.58 (95% CI); data from Välimäki et al., 2011]. Thus, we ended up with two independent replicates of areas representing either the core (Pulkkila, Rantsila) or edge-of-range (Utajärvi, Yli-Ii) areas of L. cervi. These core or edge-of-range areas were characterized either by high (i.e. high levels of snow discoloration on natural bedding sites) or by low (i.e. few or no areas of snow discoloration on natural bedding sites) infestation intensity on A. alces, respectively.

STATISTICAL ANALYSES

To evaluate possible differences between the study localities in tit occurrence, we conducted a chi-square test. We reduced observations to presence/absence data on tits as a group within a particular replicate during the 5-day experiment. Secondly, we explored whether pupae are under predation in the first place, and applied the data from the first experiment conducted in Konnevesi and Pulkkila. We tested whether the percentages of bedding sites impacted by predators vary between the two localities with a Fisher’s exact test. The analyses were performed in SPSS 15.0 for Windows.

We performed further statistical analyses with R 2.10.1 (R Development Core Team, 2009). We analysed the effects of exploitable cues that derive from the host itself (faeces) or from the host–parasite interaction (snow discoloration) with a generalized linear mixed-effect model (function glmer) fitted with Laplace approximation, as implemented in the R package lme4 (Bates & Maechler, 2009). We used binomial error distribution with logistic link function. First, we set the frequencies of eaten and uneaten pupae at the lowest level of hierarchy (the unit of 10 pupae in a particular quadrat–manipulation combination, measured 8, 24, 48, 72, and 116 h after the onset of the experiment) as the response variable (data from Rantsila). We set the incidence of snow discoloration and faecal pellets as fixed factors. We included an interaction term between the fixed factors in the model to test the possible cumulative effect of independent cues. We built the model so that it allowed random intercepts for each quadrat as well as quadrat-specific random slopes in relation to experimental manipulations, and interactions between the two.

Secondly, we tested possible spatial variation in the liability of the most prominent environmental cue (snow discoloration) by fitting a generalized mixed-effect model to the data from the study areas of Rantsila (two manipulations: only pupae; snow discoloration), Utajärvi, Yli-Ii, and Pulkkila (additional set-ups). The response variable was the frequency of eaten and uneaten pupae at a particular moment in time on a particular artificial bedding site. The fixed factors were presence of snow discoloration and natural infestation intensity (high/low). In addition, we set the continuous explanatory variable, which was time elapsed since the onset of the experiment. We defined random effects by nesting random pairs of artificial bedding sites within random study areas so that pair-specific random intercepts and random snow discoloration × time interactions were allowed. We assessed the goodness-of-fit of the model by visual evaluation of residual plots in each case.

RESULTS

We observed several tit species on both artificial and natural A. alces bedding sites in all five localities. The willow tit (Poecile montanus), the crested tit (Lophophanes cristatus), the great tit (Parus major), the blue tit (Cyanistes caeruleus), and the coal tit (Periparus ater) were the species observed. We observed tits to occur at roughly the same frequencies in each study site (tit observations: Konnevesi 100%, Pulkkila 85%, Rantsila 75%, Utajärvi 75%, and Yli-Ii 100% of all replicates; \( \chi^2 = 9.032, \text{d.f.} = 4, \ P = 0.06 \)). We did not observe any signs of small mammalian predators in the immediate vicinity of the artificial bedding sites.

BEDDING SITES IMPACTED BY PREDATION

In Konnevesi and Pulkkila, 25 and 20% of the artificial bedding sites, respectively, were impacted by predators during the 116-h trials (Table 1). The percentage of bedding sites impacted by predation did not differ statistically between the two areas (Fisher’s exact test, \( P = 1 \)). All the pupae disappeared from two bedding sites in Konnevesi and from one bedding site in Pulkkila.

HOST-RELATED CUES AND PUPAL PREDATION

There was apparent variation in the percentage of artificial bedding sites impacted by predators.
in relation to manipulation [manipulation 1 (only pupae), 20%; manipulation 2 (pupae + faecal pellets), 15%; manipulation 3 (pupae + discoloration), 100%; manipulation 4 (pupae + snow discoloration + faecal pellets), 95%]. Each of the impacted bedding sites within manipulations 3 and 4 were totally emptied of pupae, with the numbers of totally emptied bedding sites being one and three in manipulations 1 and 2, respectively. Snow discoloration on the artificial bedding sites was the only cue with a significant effect on pupal predation rate (Table 2). Snow discoloration increased the predation risk of pupae (Fig. 2). There was neither a main effect of faecal pellets on the pupal predation rate nor significant interaction between the two factors (faecal pellets and snow discoloration) (Table 2), with the latter indicating a lack of cumulative effects of various cues. The time until all pupae were consumed did not differ between the bedding sites with discoloration alone (31 h; 95% CI 16.4–45.6 h) and the sites with both discoloration and A. alces faecal pellets (33 h; 95% CI: 18.2–47.8 h).

### Table 2

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Parameter</th>
<th>Estimate</th>
<th>SE</th>
<th>Z</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pupal survival</td>
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<td>3.415</td>
<td>0.0006</td>
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<tr>
<td></td>
<td>Discoloration</td>
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<td>3.037</td>
<td>-3.629</td>
<td>0.0002</td>
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<tr>
<td></td>
<td>Faecal pellets</td>
<td>1.365</td>
<td>5.640</td>
<td>-0.214</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Discoloration × faecal pellets</td>
<td>-1.209</td>
<td>5.640</td>
<td>-0.214</td>
<td>NS</td>
</tr>
</tbody>
</table>

The model included experimental quadrat-specific random intercepts with random snow discoloration × faecal pellets interactions.

NS, not significant.

### Figure 2

Proportion of uneaten Lipoptena cervi pupae (±95% CI) on artificial bedding sites in Rantsila at 0, 8, 24, 48, 72, and 116 h since the start of the experiment, in relation to an experimental manipulation of bedding sites.

**Lipoptena cervi infestation intensity and predation rate of pupae**

The frequency of uneaten pupae changed over time across the study areas of Utajärvi, Yli-Ii, Pulkkila, and Rantsila (Table 3). In this data set, the effect of snow discoloration on the pupal predation rate was not straightforward, but depended on the prevailing L. cervi infestation intensity in natural A. alces populations. This was indicated by the third-order interactions among discoloration, infestation intensity, and time (Table 3). In the study areas where L. cervi infestation intensity was relatively low (or zero), snow discoloration only moderately increased the pupal predation rate on artificial bedding sites (Fig. 3A) compared with the apparent increase in the highly...
infested areas (Fig. 3B). Moreover, the percentages of impacted bedding sites with no snow discoloration in the study areas showing a low infestation intensity (Utajärvi, 13%; Yli-Ii, 25%) were of the same magnitude as in the heavily infested areas (Pulkkila, 19%; Rantsila, 20%), but there was a difference concerning discoloured bedding sites (Utajärvi, 56%; Yli-Ii, 38%; Pulkkila, 94%; Rantsila, 100%).

**DISCUSSION**

Our results show that the predation pressure from tits on *L. cervi* pupae is notable during winter. Thus, our data are among the first in the Northern Boreal region to demonstrate that avian predation may affect the population dynamics of ectoparasites feeding on large mammals. This result should be acknowledged in future models when evaluating *L. cervi*–*A. alces* interactions in an evolutionary time scale. The reddish brown snow discoloration on host bedding sites, indicating high *L. cervi* infestation intensity (see Kaunisto et al., 2009; Välimäki et al., 2011), and consequently a rewarding feeding site for tits, seems to increase the risk of pupal predation.

The assumption that avian species are potential predators of *L. cervi* holds true, as we witnessed several tit species (*Poecile montanus*, *Lophophanes cristatus*, *Parus major*, *Cyanistes caeruleus*, and...
*Periparus ater* foraging on pupae on either natural or artificial bedding sites. Tits are highly adaptable predators that can rapidly respond to the occurrence of a new abundant resource, especially invasive or suddenly increasing insect populations (Pimentel & Nilsson, 2007). During the winter, the tit guild forages frequently in mixed-species flocks (Suhonen & Nilsson, 2007). During the winter, the tit guild forages frequently in mixed-species flocks (Suhonen et al., 1994; Dolby & Grubb, 2000), which may explain the fast and complete disappearance of the pupae from discoloured bedding sites once discovered. Indeed, interspecific interactions with other insectivorous birds indicating high food availability can increase predation on a specific prey (e.g. Forsman, Hjernquist & Gustafsson, 2009). Native avian predators can benefit for the expansion of an invasive insect in some cases (see e.g. Barber et al., 2008). In addition to the species observed, a few other passerine species foraging frequently on the ground and on snow in the Northern Boreal forests may have been involved in the predatory guild (see Alatalo, 1980). We stress that in this study other potential predators such as voles, shrews, and mice are unlikely to be responsible for pupal disappearance above the snow layer. This is because we did not observe any signs of those species in the immediate vicinity of the artificial bedding sites, and small mammals spend most of the winter beneath the snow anyway (e.g. Hansson & Henttonen, 1985; Aitchison, 1987).

The *A. alces* faecal pellets studied as a possible cue for predators did not increase the risk of pupal predation. The lack of response is understandable, as host faeces may not reliably indicate the presence of prey items on a bedding site, unlike the commonly observed snow discoloration that derives from the direct host–parasite interaction. Indeed, the reported reddish brown snow discoloration of *A. alces* bedding sites (see Kaunisto et al., 2009) significantly increased the probability of pupae being predated upon. In addition to being a visual cue, discoloured snow may offer olfactory stimuli. Avian olfaction is still poorly understood, but it may be a more important sense than is generally believed (see Mäntylä et al., 2008; Steiger et al., 2008).

An increase in pupal predation with increasing infestation density and degree of snow discoloration may suggest an anti-apostatic selection, meaning over-predation on rare prey types at high densities rather than an apostatic selection (over-predation on common prey types) (see Allen & Anderson, 1984). In the natural bedding sites of *A. alces*, *L. cervi* pupae can most probably be observed on the spots of discoloured snow, near the neck and back regions of a bedding site (S. Kaunisto & P. Välimäki, pers. observ.). The clumped distribution of pupae on the bedding sites is caused by the aggregation of adult *L. cervi* on the host, favouring the neck and back regions where they feed on blood and reproduce (see Paakkonen et al., 2010). The clumped distribution is probably the most common dispersion pattern of forest insects (Coulson & Witter, 1984), although some of *L. cervi* pupae may drop off a host randomly outside the bedding sites. There is disagreement about whether avian predators can limit insect numbers (Roland, Hannon & Smith, 1986). However, birds can opportunistically exploit aggregations of insects, and even at lower insect densities avian predation can inflict notable mortality on an insect population (e.g. Schultz, 1983; Roland et al., 1986).

*Lipoptena cervi* pupae that drop off a host with relatively low infestation intensity are likely to escape predation. Most of the artificial bedding sites without snow discoloration (80%) remained undetected during a monitoring period of 116 h. This also held true in the study areas where the infestation intensity of *L. cervi* within the natural *A. alces* population was relatively high (Konnevesi, Pulkila, and Rantsila) (*N* = 92). Hence, it can be assumed that black pupae against a white background do not provide enough information for tits, and that clean, non-coloured bedding sites are merely foraged upon incidentally. Alternatively, tits can discover non-coloured bedding sites, but a lack of information on the profitability of the site leads the predators to reject it. Even when clean bedding sites were found, predation on them took place at earliest after 48 h. Weather conditions may change even within a day in winter, when pupae are exposed to predation for relatively short times until being covered by snow. Consequently, a time lag of 48 h is enough to decrease predation risk considerably.

In the heavily infested study areas (Rantsila and Pulkila), 39 and 63% of discoloured bedding sites (*N* = 56) were entirely emptied by predators within 8 and 24 h, respectively. According to these results, *L. cervi* pupae were soon consumed by predators once they had been discovered. Hence, relatively low pupal loss in the two treatments without snow discoloration indicates that potential predators had not discovered these bedding sites, rather than the *L. cervi* pupae being unpalatable prey. It has been shown that tits forage according to the optimal foraging theory (see Krebs et al., 1977; Koivula, Rytkönen & Orell, 1995; Brotons, 1997). For example, tits can adjust their foraging behaviour on the basis of prey density and quality (e.g. Naef-Daenzer, Naef-Daenzer & Nager, 2000). Potentially, tits may use discoloration to evaluate expected nutritional gain and adjust their time expenditure within a particular bedding site adaptively. In line with this scenario, a group of pupae deposited on experimental bedding sites without snow discoloration were relatively frequently only partially
consumed (70% of impacted clean bedding sites), even in the heavily infested areas where discoloured beddings sites were regularly emptied as a whole.

The avian predators may have learned to associate snow discoloration with a potential food source in areas where overwintering A. alces and L. cervi are abundant. Birds may also have an inherent tendency to be attracted to signals like blood containing UV-fluorescent molecules (see Bennett & Cuthill, 1994; Viitala et al., 1995; Church et al., 1998). Our results, however, do not support this latter possibility, as discoloration did not have such a large effect on pupal predation in Yli-Ii and Utajärvi, where the overwintering A. alces populations were not heavily infested, resulting in a relatively low degree of discoloration on natural bedding sites. Our results indicate that predator response to snow discoloration is likely the result of phenotypic plasticity, where individuals fine-tune their behaviour to match the prevailing environment (e.g. Carlsson et al., 2009). There are several mechanisms by which native predators may become better in exploiting novel species as prey, including the formation of a search image, associative learning and social transmission (e.g. Krebs & Davies, 1993). The observed pattern may also result from the spatial variation in tit abundance that was not rigorously assessed, and thus some caution is warranted. It is worth noting, however, that tits were observed in the same frequencies within each study site, and the most parsimonious explanation for equal predation upon pupae deposited on non-coloured bedding sites would be equal predator density.

We provide the first estimations about the magnitude of predation on the novel invading prey, L. cervi, in the Northern Boreal region during winter. Our results share a high resemblance to natural conditions, as each experiment was conducted in the field with natural predator guilds and the extent of experimental manipulations fell within the natural variation of A. alces bedding characteristics in areas with moderate L. cervi infestation intensity. As 34.7% of all pupae used in the experiments were predated upon, even within a relatively short time interval, we argue that pupal predation during winter can be of importance. However, avian predation is unlikely to prevent the parasite from spreading into new areas, because predation on the bedding sites of hosts with low infestation intensity, and thus without remarkable snow discoloration, seems coincidental and ineffective. In addition, the predation risk for an individual pupa that drops off of a moving cervid host is likely to be marginal, unless avian predators learn to follow the movements of parasitized hosts (see, e.g., Haarläv, 1964; Samuel, Mooring & Aalangdong, 2000). In general, the parasite infestation intensity is likely to be low during the early phase of invasion, but later on, when the parasite population has established properly, tit predation could strongly affect the local population dynamics. This would occur if birds learn to take advantage of cues that derive from the host–parasite interaction. Even then, hosts that harbour parasites with low infestation intensity could ensure the sustainability and spread of the parasite population. Before firm conclusions on the importance of pupal predation could be drawn, analyses of tit diets and on-host mortality of L. cervi are warranted. Nevertheless, pupal predation should not be ignored when evaluating the three- or two-way interactions among L. cervi, A. alces, and the predatory tits.

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REFERENCES

Bates D, Maechler M. 2009. lme4. Linear mixed-effects models using S4 classes. R Package, Version 0.999375-32. Available at: http://cran.r-project.org/web/packages/lme4/index.html


Supporting information

Additional supporting information may be found in the online version of this article:

**Appendix S1.** A schematic view of the experimental designs used at the different study sites.

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