Ficedula hypoleuca hemoglobin levels in lead contaminated areas

Is bird health affected by invertebrate community composition and abundance?

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Abstract

Mining is a widespread industrial activity that in many cases, via mining wastes, leads to altered concentrations of metals in close vicinity to the mining activities. Metals in mining waste can have high toxicity and may persist in environments for long time periods. The presence of metals, such as lead (Pb), is known to contaminate and cause damage to nearby organisms and ecosystems. Birds are at risk of metal contamination and, since they are predators high up in the food chain, may face accumulation of metal in tissue over time, via consumption of contaminated prey. Small passerine species, such as the pied flycatcher *(Ficedula hypoleuca)*, are suitable for studying metal contamination as they are ubiqutious, and high up in the food chain. Pied flycatchers feed exclusively on invertebrates, making the abundance, quality, and potential contamination of invertebrates interesting to study with regard to the health of the birds. Studies have shown that hemoglobin (Hb) levels in young pied flycatchers are reduced by high background levels of Pb. This may be linked to prey availability and quality, as invertebrates are known to alter their composition, and contain higher Pb concentrations in Pb contaminated areas. Here, I investigated how invertebrate abundance and community composition, and pied flycatcher Hb concentrations (i.e. health), in reference and Pb contaminated areas, were related to each other. Invertebrate traps were set and sampled twice during the summer of 2018 to provide invertebrate data to the study. Bird Hb levels was acquired by taking blood samples from nearly fledged chicks in birdhouses placed in the different areas. I found a potential trend towards higher Hb levels in reference areas ($p=0.110$), suggesting that bird health is reduced by the presence of Pb, but this could not be explained by differences in invertebrate community composition or abundance ($p>0.05$). Hence, based on this study, high Pb concentrations in the soil does not directly, or indirectly via potential impacts on the prey community composition, influence the health of pied flycatchers.

Key words:
Metal contamination, pied flycatcher, hemoglobin, invertebrate abundance, invertebrate composition

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1 Introduction

1.1 Background

Metal contamination is a threat to ecosystems, and one major source of metal emissions is the mining industry (Janssens et al. 2003, Berglund et al. 2011). Mining has been present for centuries, and the metal waste can persist in environments long after abandonment, causing damage to nearby ecosystems (Johnsson 2003). Metal waste can cause severe ecosystem damage due to high toxicity and extensive industrial use, leading to high concentrations of toxic heavy metals (Baird and Cann 2012). The point-source pollution of heavy metals alters species composition or reduces the abundance of local plants and invertebrates, and can also affect higher organisms, as heavy metals are known to bioaccumulate in the food chain (Kiikkilä 2003). One example of a heavy metal contaminant is lead (Pb), which is toxic in most chemical forms and therefore harmful to the organisms within an ecosystem (Eisler 1998). Pb can accumulate in soils and the Pb uptake of plants from the soil allows Pb to ascend in the food chain, posing threats to organisms at the top of the food chain, such as birds, fish, and mammals (Gall et al. 2015, Assi et al. 2016).

Birds are one organism group at risk with regard to metal contamination. Metal uptake for birds mainly occur through feeding on contaminated prey. Heavy metal concentrations in bird tissues can remain high long after nearby emissions are reduced, and the danger birds face is an accumulation of metal over time (Berglund and Nyholm 2011). Due to the accumulation of heavy metals in birds, they may be a good indicator for measuring effects of metal exposure on food webs. More specifically, small passerine species make suitable indicators as they are ubiquitous, well studied, and high up in the food chain (Janssens et al. 2003). One species of a passerine bird that has been proven to be affected by metal pollution, including Pb, is the pied flycatcher *(Ficedula hypoleuca)* (Eeva and Lehikoinen 1996, Berglund et al. 2011, Eeva and Lehikoinen 2015). Pied flycatchers are opportunistic foragers and have a wide diet of invertebrates (Eeva et al. 2005). Diet choice may affect sensitivity to metal pollution, but in a comparison between pied flycatcher, opportunistic hunter, and great tit (*Parus major*), which is a specialist, it was found that both species showed equal concentrations of Pb in tissue, thus suffering from equal exposure to Pb contamination (Belskii and Belskaya 2013).

Previous studies have shown that pied flycatchers have higher tissue concentrations of Pb in contaminated areas, even long after industrial emissions have stopped (Berglund et al. 2009). Since pied flycatchers are sensitive to Pb accumulation they could provide risk-assessment information in areas with high levels of Pb waste, according to Berglund et al. (2011). The breeding success for pied flycatchers, and more specifically hemoglobin (Hb) levels in young birds, is affected by high background levels of Pb (Berglund et al. 2010). Low concentrations of Hb seems to be related to high Pb contamination (Berglund et al. 2010), and low Hb levels can lead to anemia (Rodak et al. 2007). Anemia lowers the blood's ability to transport oxygen, leading to weakness and susceptibility to disease (Rodak et al. 2007). Moreover, Pb affects bird health by altering behaviour, nervous systems, and reducing reproduction (Burger and Gochfeld 2000, Janssens et al. 2003, Berglund et al. 2010).

The pied flycatcher feeds on invertebrates, and invertebrate composition is known to be altered, and their abundance reduced, when exposed to metal pollution (Kiikkilä 2003). Thus, not only do pied flycatchers suffer from direct metal exposure via food intake, but also indirectly, from changes in their diet as invertebrate communities change due to metal contamination. Direct effects such as food quality may influence the health of predators, such as the pied flycatcher. It has been shown that food quality can decrease in metal contaminated areas, as metal concentrations in invertebrates were significantly higher than in reference areas (Dauwe et al. 2003, Eeva et al. 2005, Belskii and Belskaya 2013). More specifically, spiders had the highest concentrations of Pb, followed by beetles and larvae (Eeva et al. 2005). Moreover, invertebrate composition was affected in metal contaminated areas due to higher mortality of sensitive species, leading to advantages for more resistant species and a change in the community structure, indirectly affecting predators on invertebrates by altering their diet (Kiikkilä 2003).

1.2 Aim

This study focused on the health of the pied flycatcher, by investigating hemoglobin (Hb) concentrations in nearly fledged chicks and the community structure of its main food source, invertebrates, in forests close to Laisvall, Norrbottens län, Sweden. The area has a history of Pb mining, which has led to high background levels of Pb in the soil. The aim of this study was to 1) compare pied flycatcher Hb levels between metal contaminated and reference forests, 2) compare the invertebrate abundance and community composition between metal contaminated and reference forests, and 3) see if pied flycatcher health can be linked to food quality and availability. The hypotheses were i) that pied flycatcher health will be higher in the reference forests ($p<0.10$; one-tailed hypothesis), ii) that invertebrate abundance and community composition would differ between the areas, and iii) that Hb levels in young birds can be explained by prey availability and composition.

2 Method and material

2.1 Method

Invertebrate samples were collected in three reference and three contaminated forests, using five pitfall traps and three deposition traps within each forest. The pitfall traps were cup shaped with a diameter of 9 cm, while the deposition traps were square (18x18 cm). Both trap types were filled with a mixture of 50% propylene glycol and 50% water to preserve samples, and a few drops of detergent to break the surface tension. All traps were sampled twice during summer; traps were deployed during 13-15/6, 2018, and the field work of collecting samples was conducted 26-27/6 and 02-04/7 in 2018. The samples were later examined in the lab and determined to different categories, i.e. terrestrial insects, aquatic insects, ants, spiders, and big non-diet (for pied flycatchers) beetles (*Carabidae* and *Cetoniidae*). Each individual trap was sorted into these categories in separate samples. Invertebrate samples were later freeze dried, and dry weight for each sample was measured in grams with a precision of three decimals.

Hb concentrations (g/liter) were acquired by analyzing blood samples from nearly fledged chicks in birdhouses placed in the forests (approved by the Swedish EPA and the Committee for Ethical Review).

2.2 Study site

The study was conducted in Laisvall and Gauto, Norrbottens län, Sweden (figure 1). Laisvall has a history of lead mining and high background levels of Pb in the soil making it ideal to study effects of Pb contamination. Gauto is located further upstream Laisälven and has normal background levels of Pb. Both areas have similar ecology and the main difference is the metal exposure (Berglund et al. 2010). Pb soil concentration samples were available for contaminated forests but not for reference forests. However, available data from lakes in each area showed 10 times higher Pb concentrations in contaminated lakes. Furthermore, the concentrations of Pb in soils was approximately 100 times greater in contaminated forests than in sediments of reference lakes.

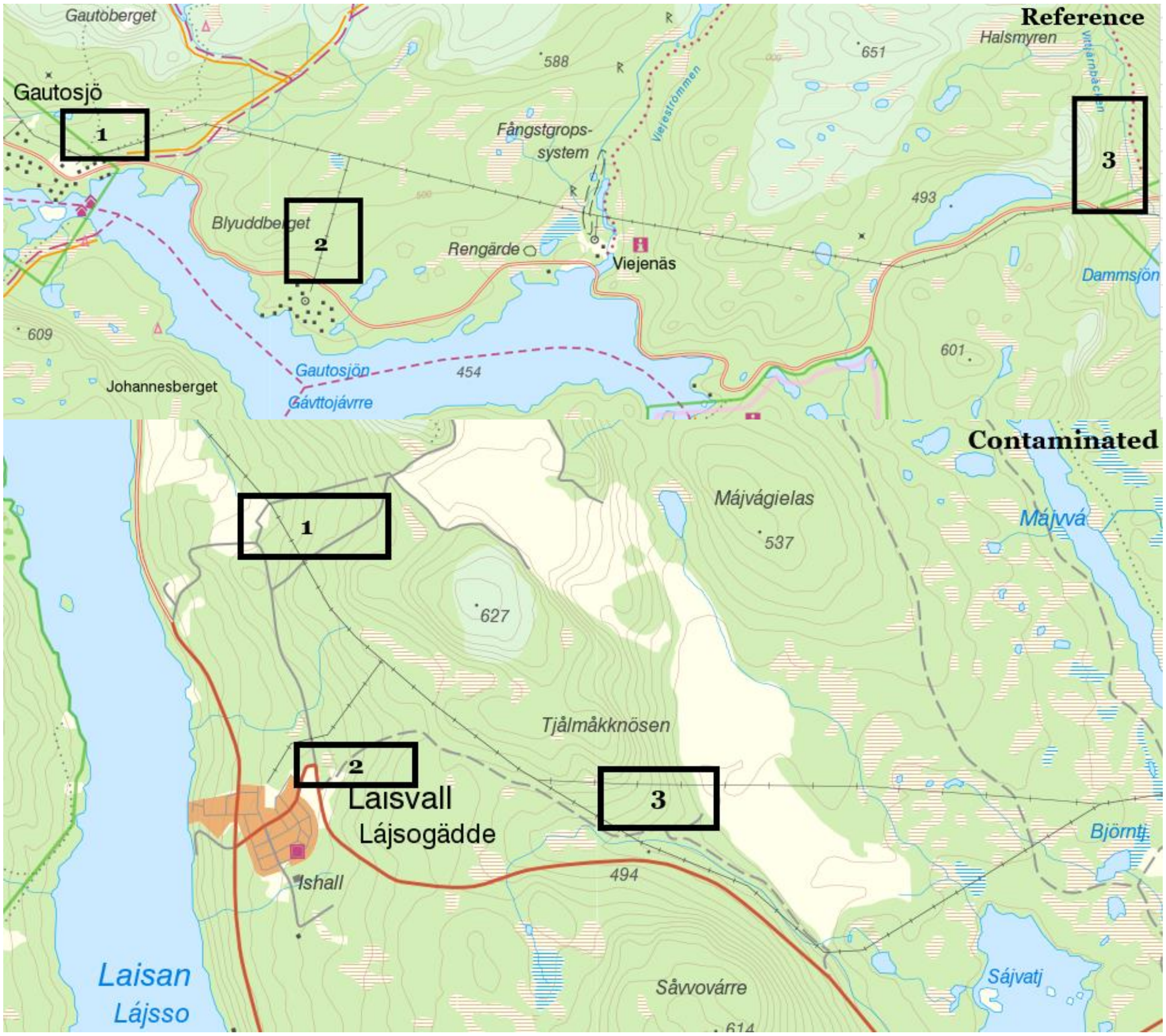

Figure 1. Maps of reference (top) and contaminated (bottom) areas, with each replicate marked from number 1-3 in both areas (Lantmäteriet 2018).

2.3 Data analysis

The data were processed using Excel. The invertebrate data showed large variations around means, likely because only three replicates of reference and contaminated areas were compared. Significance was tested using the robust analysis of variance (ANOVA) that can withstand some deviance from normal distribution. Since the traps were sampled twice, and the amount of days a trap was active differed, the amount of invertebrates caught per day was compared for both sampling periods. The categorization of insects and spiders was necessary to avoid extreme values from ants, spiders, and non-diet beetles (*Carabidae* and *Cetoniidae*). The non-diet heavier beetles were later removed from the analyzed data as they are not in the pied flycatcher diet. Furthermore, the 'terrestrial insect' category consists of insects that have all their life stages in terrestrial environments (exceptions explained above). The 'aquatic insect' category consists of all insects having at least one aquatic life stage. For instance, *Diptera* species belonging to the *Simuliidae and Chironomidae* families have their larval stage in aquatic environments and spend their adult life in terrestrial habitats, thus occurring in terrestrial traps.

The Hb level in nearly fledged chicks was tested using a standard t-test in Excel, assuming a one-tailed hypothesis with higher Hb levels in reference forests. The data proved to be normally distributed after log-transformation.

Regression analyses were conducted to compare invertebrate proportions (% of each group as a proportion of the total abundance) with Hb concentrations, for each invertebrate category, to investigate potential relations between the Hb and the invertebrate data. The regression analysis was done for pitfall traps and deposition traps separately.

3 Results

3.1 Time effect and invertebrate abundance

For pitfall traps, there was no difference in the amount of invertebrates caught per day between sampling 1 and 2 ($p>0.05$; figure 2). However, there was a significant difference between the amount of invertebrates caught per day between reference and contaminated forests ($p=0.035$), with higher numbers in contaminated forests (figure 2).

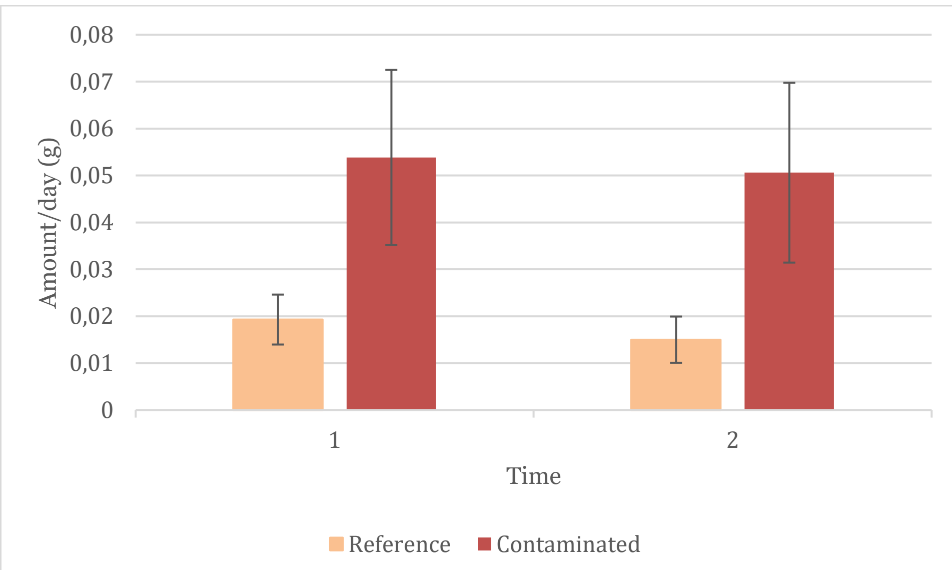

Figure 2. Invertebrates caught daily in pitfall traps for both sampling periods (time) in reference and contaminated forests. Error bars show ±1 standard error.

For deposition traps, there was no significant difference between reference and contaminated forests, and no differences between sampling events ($p>0.05$), but there was a significant interaction term ($p=0.011$), with a higher amount of invertebrates in the reference sites only during the second sampling period (figure 3).

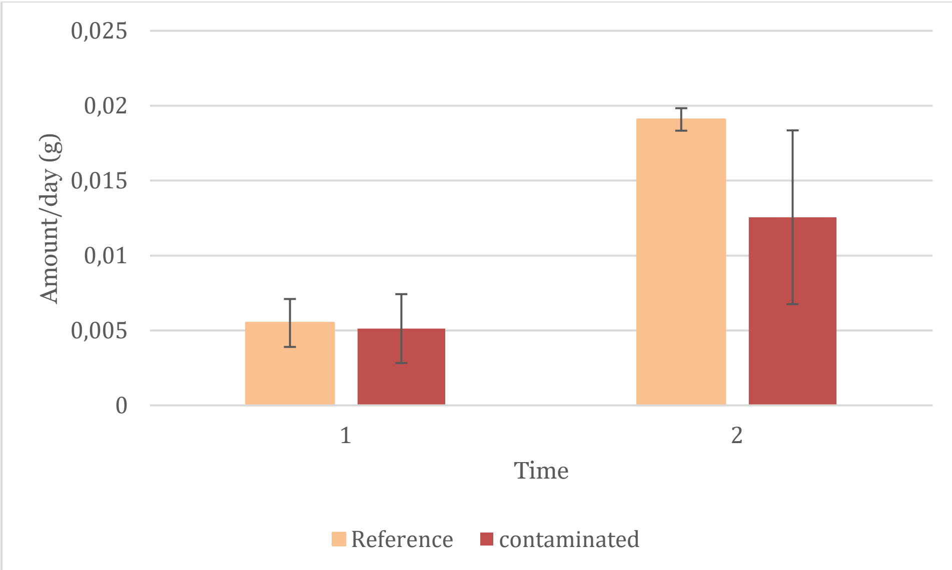

Figure 3. Invertebrates caught daily in deposition traps for both sampling periods (time) in reference and contaminated forests. Error bars show ±1 standard error.

3.2 Invertebrate community composition

Further, for pitfall traps, the composition of invertebrates differed between reference and contaminated forests ($p=0.017$), but only for certain groups of invertebrates (i.e. interaction term: $p=0.009$), while there was no effect of sampling time ($p>0.05$) (figure 4). More specifically, spiders showed higher proportions in the reference areas than in the contaminated areas, whilst the opposite was true for ants (figure 4). The categories 'aquatic' and 'terrestrial insects' showed no significant difference within their category between contaminated and references areas.

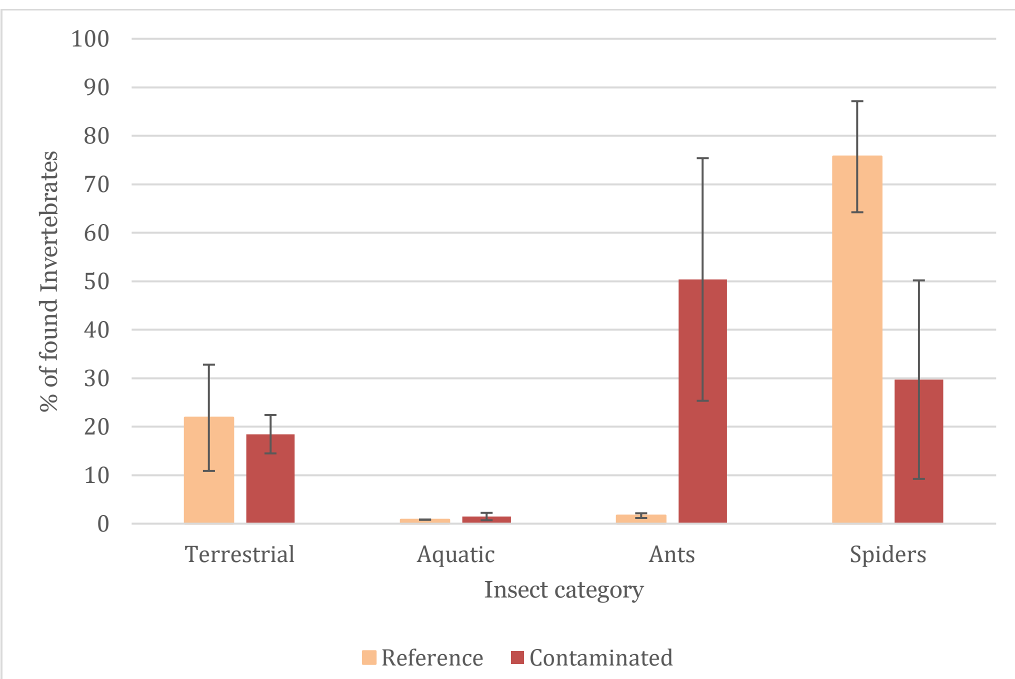

Figure 4. Pitfall trap proportion of invertebrates caught (in weight) in reference and contaminated forests. Error bars show ±1 standard error.

Since collections in the deposition traps differed significantly between sampling periods (figure 3), the two periods were analyzed separately for community composition. For both sampling periods (P1 and P2), no difference was found in the proportion of invertebrates between reference and contaminated forests (Figure 5). However, there were significant differences among the proportions of the invertebrate categories ($p<0.001$ for P1 and $p<0.001$ for P2) (figure 5), as 'terrestrial insects' had the highest abundance for both sampling periods in both areas.

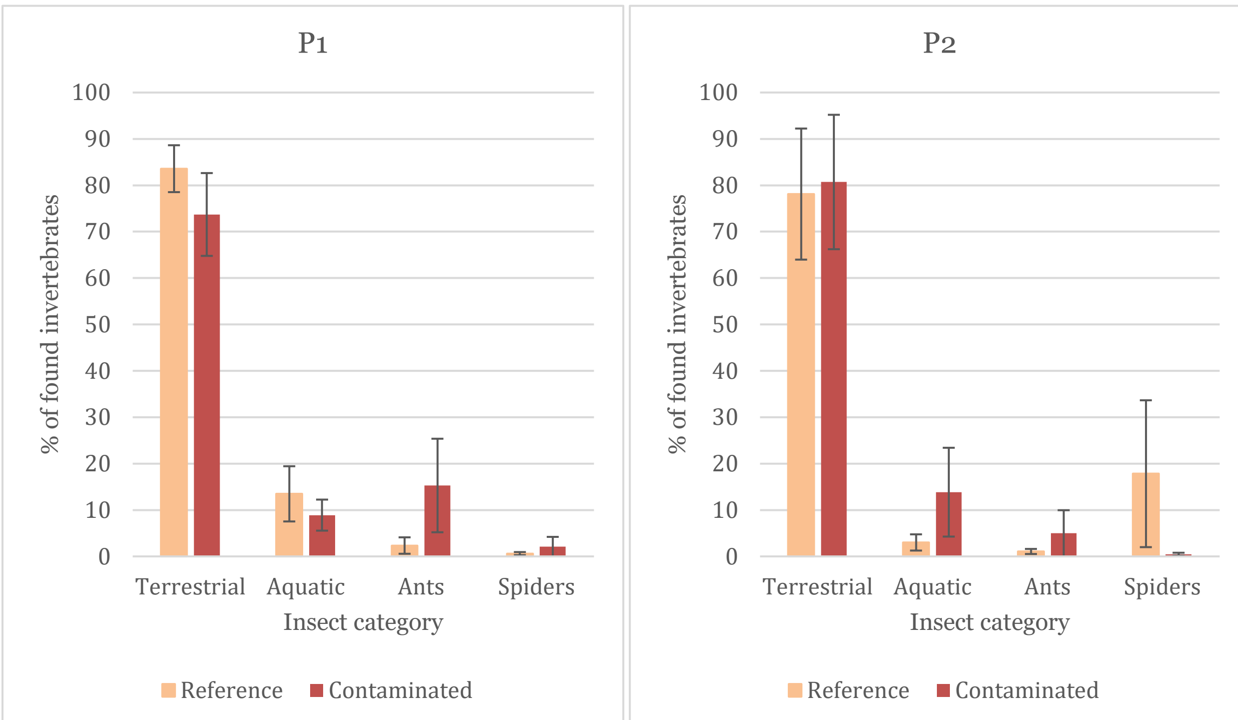

Figure 5. Deposition trap proportion (in weight) of invertebrates caught in reference and contaminated forests, for both sampling periods, P1=period 1, P2= period 2. Error bars show ±1 standard error.

3.3 Bird hemoglobin concentrations

The Hb levels in nearly fledged chicks proved to be close to significantly different between the two areas ($p=0.110$) (figure 6), i.e. there was a trend for higher Hb levels in the reference areas.

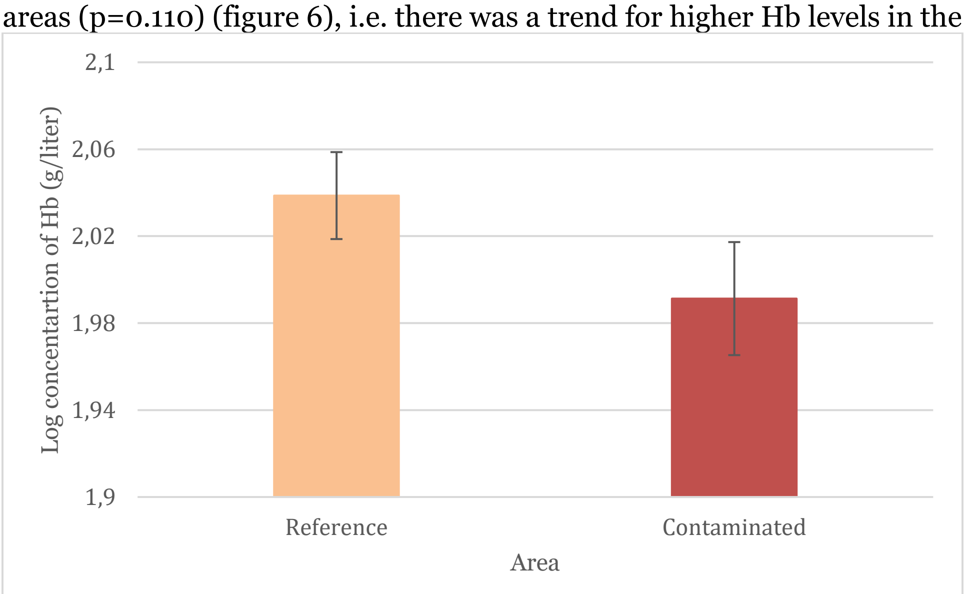

Figure 6. Log hemoglobin concentrations in nearly fledgling chicks in reference and contaminated forests. Error bars show ±1 standard error.

3.4 Regression analyses

No significant relationships between Hb concentrations and proportion of any specific invertebrate category could be found, neither for pitfall nor deposition traps.

4 Discussion

4.1 Hemoglobin levels and bird health

Previous studies have shown that hemoglobin levels are reduced in metal contaminated conditions (Nyholm 1998). Heavy metals interfere with the process of creating enzymes and proteins needed to produce hemoglobin. Pb specifically interferes with the creation of globin which is an essential compound of hemoglobin (Adolfo and Garnica 1981). In my study, it is difficult to say anything about bird health with certainty just by looking at Hb concentrations, since the result was only close to significant, but there is a tendency towards higher hemoglobin values in the reference area, which is what could be expected based on previous studies. The result might have been significant ($p<0.10$, based on the one-tailed hypothesis) given more samples and replicates. Having more replicates was, however, not possible due to time constraints. Adding more aspects of bird health to the study, such as Pb tissue concentrations and chicks survivability, would give additional information to investigate the hypothesis of better health in reference areas, but was also limited by time constraints. Nevertheless, the result might still have been non-significant, even with better replication. One explanation for a non-significant Hb concentration result could be that more prey was available in the metal contaminated areas. The invertebrates close to contaminated sites generally have higher heavy-metal concentrations than those living in reference areas, which should be negative to the health of birds breeding in contaminated areas (Dauwe et al. 2003, Eeva et al. 2005, Belskii and Belskaya 2013). However, if the food availability is high, it might compensate for poor-quality diet, and bird health could therefore be higher than expected in contaminated areas. Furthermore, the highest concentrations of Pb in invertebrates (in contaminated areas) is typically found in spiders (Eeva et al. 2005), and spiders proved to be proportionally smaller in invertebrate community composition in contaminated areas compared to reference areas. As such, positive effects of high prey availability might obscure expected negative effects of higher prey metal concentrations, thus leading to better-than-expected bird health in contaminated areas.

4.2 Invertebrate abundance and community composition

Since traps were sampled twice, time had to be tested as a factor to get comparable results. Time did not affect catches in pitfall traps, probably since the abundances of the most common catches in pitfall traps (spiders, ants, and small terrestrial beetles – mostly *Staphylinidae*), had stabilized by the time of the first sampling. Deposition traps caught more invertebrates in both reference and contaminated forests during the second (later) period (figure 3), which is most likely explained by an increased hatching of flying terrestrial insects, mostly terrestrial diptera (figure 5) (keep in mind that terrestrial and aquatic insect categories only refer to their life stage history, flying insects are common in both categories). Since timing proves to alter the amount of invertebrates in deposition traps (i.e. mostly flying insects), more sampling periods could be added for deposition traps to acquire mean values of invertebrate data for the whole pied flycatcher breeding period, to properly investigate differences between reference and contaminated forests.

When analyzing the invertebrate data a few things stand out. Most surprising might be that contaminated forests had significantly higher amount of invertebrates than reference forests for pitfall traps (figure 2). I was expecting the reference forests to have higher abundance of invertebrates due to lower Pb contamination. One explanation could be a higher level of predation in the reference forests. Although this is rather speculative, the fact that spiders were dominating in the reference forests, while their proportions were significantly lower in the contaminated forests, could support predation as one factor that determine overall invertebrate abundance, since they prey on other invertebrates (Bucher et al. 2015). Higher proportions of predators could also indicate higher productivity of prey, meaning that productivity may be higher in the reference area even if the abundance is higher in the contaminated area. With that said, according to my findings, food availability should not limit pied flycatcher health in contaminated areas.

The significant differences between invertebrate categories came as no big surprise, as previous studies have shown that metal contamination can alter species composition (Kiikkilä 2003). Ants dominated the contaminated forests whilst spiders are the most abundant in reference forests. Hence, spiders seem to suffer from metal contamination the most, possibly due to its role as a predator and high position in the food chain. Eeva et al. (2005) have shown that spiders had the highest concentration of Pb of all invertebrates in metal contaminated areas. Ants seem to be highly favoured in metal contaminated areas for reasons unknown. According to one study by Silverin and Andersson (1984), breeding pied flycatchers has a diet high in ants (25% of total diet). Hence, if bird predation is a factor controlling invertebrate community composition, one hypothesis is that there are more birds present in the reference forests, limiting the amount of ants in those areas. However, if ants are such an important prey for nestling pied flycatchers, there should be no shortage of food for birds that breed in contaminated areas and it might contribute to better (than expected) health for birds breeding in the contaminated area. The study by Silverin and Andersson (1984), was not conducted in Laisvall, however, and since pied flycatcher diet change depending on the invertebrate composition in their habitat (Belskii and Belskaya 2003), it might not be comparable to nestling birds in Laisvall forests.

4.3 Conclusions

Hb concentrations, i.e. health, in young birds showed a tendency to be higher in reference areas. Invertebrate abundance and composition proved to differ between reference and contaminated areas, but there were no clear associations between bird health and prey availability and invertebrate community composition. More replicates might have yielded different results, as three replicates could lead to poor predictions of means. Future studies may want to focus on more aspects of bird health as well as more comprehensive replication. Since time period was important for the abundance of some invertebrate categories, it might also be interesting to study if Hb concentrations vary across time. Another aspect to consider is the level of predation, both from the birds and the top invertebrate predators, as it might explain some of the results found in invertebrate abundance and composition. Furthermore, investigating prey quality more closely by looking at Pb concentrations in each invertebrate category in different areas, could have given a greater insight into how pied flycatchers may be influenced by mining waste contamination.

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