DETECTABILITY OF INSECTS IN MALAISE TRAPS

Assessing insect detectability in Malaise traps through High-Resolution Sampling, and development of a novel marking technique

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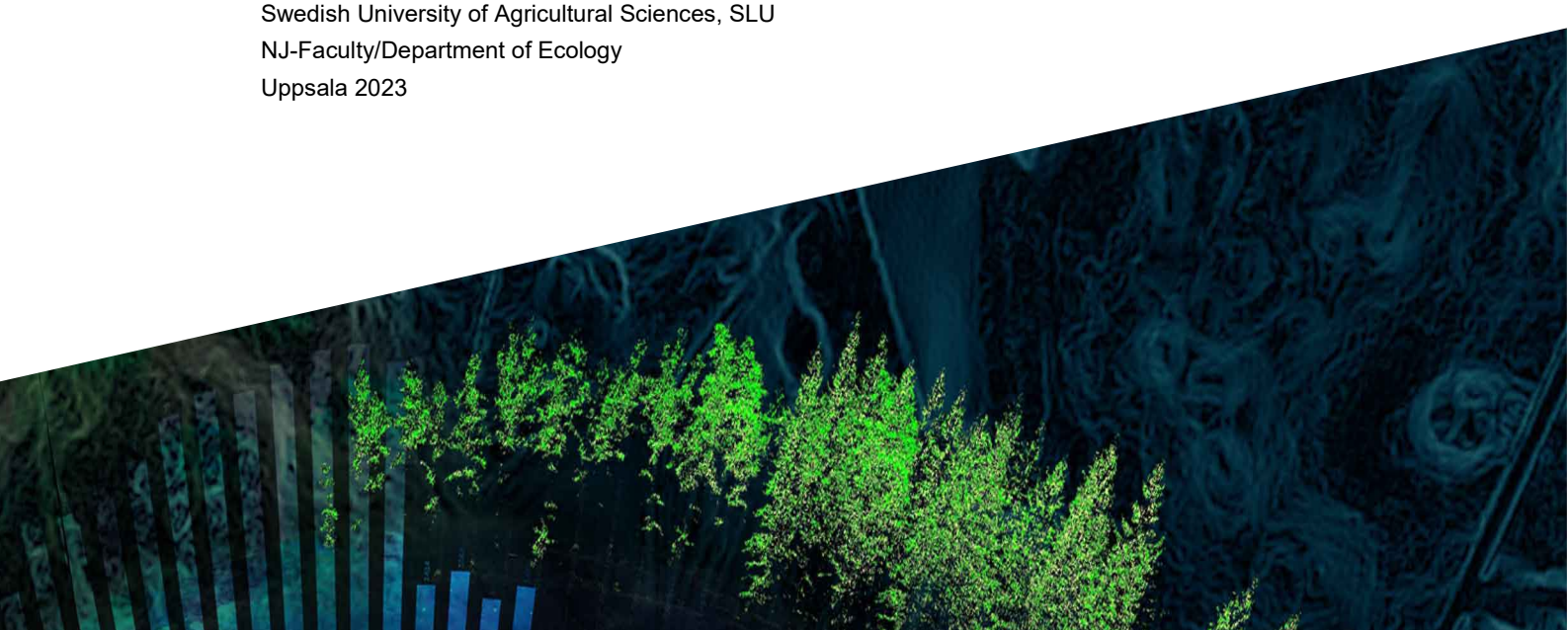

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Abstract

Malaise traps are extensively used in insect surveys and studies, yet the detectability of insects in relation to the traps remain unknown. Detectability is the odds that a taxon ends up in a trap, given that the taxon is present in the area around the trap. The detectability of a taxon depends on three main factors – Population size of the taxa, its activity, and its movement patterns. In this study, I aimed to find how weather influence the activity and thus detectability of insects in Malaise traps, and to develop a marking technique for surveying insect movement patterns around the traps. To achieve my aim of developing a novel marking technique, I tested the effect of fluorescent dusts on survival and flight ability of insects, concluding that the dusts showed no overall negative effects. I then developed a passive marking device of insects in Malaise traps, using the fluorescent dusts as marking mediums. I re-released marked individuals in a forest habitat, inside a network of Malaise traps, and emptied the traps each day. The recapture rates of marked individuals were 4.5% and the technique deemed an effective method for passively marking insects. Furthermore, to understand how weather influences activity and subsequent detectability, I conducted High-Resolution Sampling. I emptied 24 Malaise traps every second hour for five days, recording temperature, cloud cover, wind speed and relative humidity at each sampling event. This data was used to investigate how these variables affect insect activity, and in what directions. Increased temperature increased abundances of insects. Increased wind speed and cloud cover lowered abundances. Relative humidity had no effect on abundances. Furthermore, high wind speeds, and to some extent temperatures, subset the sampled community by favouring insects with smaller wing area than expected by the normal wing-to-body ratio. However, the effect of each environmental factor was small. In conclusion, this study shows that Malaise trap samples are robust against fluctuations in weather, and that the novel marking technique presented is an effective method for marking minute insects.

Keywords: Malaise trap, activity, movement, weather, mark-recapture, detectability, insects

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Introduction

History of the Malaise trap

In 1937, René Malaise published the first prototype of a novel insect trap (Malaise 1937), a tent-like structure that passively caught flying insects. The insects would hit the tent wall and walk upwards toward the sunlight, ending up in a collecting bottle placed at the top of the structure. Since the design relied on insects clinging to the tent wall when encountering it and exhibiting a positive phototactic behavior, it was most effective in catching small, lightweight insects in groups such as *Hymenoptera* and *Diptera*. In contrast, more heavy insects in the order of *Coleoptera* would fall to the ground when they encountered the tent wall, and very advanced flyers like *Odonata* could simply avoid the structure completely (Matthews & Matthews 2017).

This novel trap design did not gain much attention at first. It was not until Henry Townes published an article on the assembly of a refined version of what had then become known as a *Malaise trap* that the practical application of these traps became widespread (Townes 1962). While Townes' new design was meant to increase catch rates of *Ichneumonidae*, he also stated its effective use in catching other Hymenopterans, as well as Dipterans and Lepidopterans.

Since the development of Townes improved model, Malaise traps have been used in countless studies for a multitude of purposes such as in studies of local biodiversity (Ohsawa 2010, Janzen *et al.* 2020), or in studies mapping the insect faunas of entire countries (Steinke *et al.* 2017, Karlsson *et al.* 2020). They also feature predominantly in highly debated subjects such as the potential insect apocalypse (Hallmann et al. 2017). The Malaise trap has proven particularly effective in quantitative studies, as a productive trap can sample over 250 individuals per day, reaching as high as 750 individuals in one day during ideal conditions (Matthews & Matthews 2017). Today, there are countless variations and tweaks to the Malaise trap design. In general, 90% of the sampled individuals consists of species from the Orders *Diptera, Hymenoptera, Lepidoptera* and *Hemiptera*, with *Diptera* alone comprising more than half of the samples individuals over a season (Matthews & Matthews 2017).

The utilization of Malaise traps has been historically hampered by the large amount of taxonomic knowledge and time needed to identify the catches. However, the recent advent of meta-barcoding revolutionized the use of Malaise traps for bulk sampling (Taberlet *et al.* 2012, Yu *et al.* 2012). Despite Malaise traps' easy, cheap and effective use for insect sampling, few studies have attempted to critically evaluate the samples in relation to actual species' communities (although see Fraser *et al.* 2008, Steinke *et al.* 2021).

Insect detectability

To relate the catches of Malaise traps to species abundances and insect community compositions in the surrounding landscapes, a few concepts and terms must be established. As a passive device, a Malaise trap will catch a fraction of the insect species and individuals present at the sampling site. (This excludes the species which actively avoid the traps; see above). We call the chance of being sampled *detectability*. Williams (1940) claimed that *"The number of insects captured in a trap per unit time is a function of the local population size multiplied by its level of activity".* Williams, however, was working with light traps which actively attracts insects. In contrast, Malaise traps samples insects that encounters the trap by random chance. When studying detectability in relation to Malaise traps, one must thus also account for the possibility that an active insect never flies into the trap. The total number of individuals one could sample is equal to the total population sizes in the area. Weather might, however, cause only a subset of those to be active during the sampling period. The movement patterns of those active insects will then decide how likely they are to encounter a trap. The detectability of a taxon in a Malaise trap is thus dependent on these three confounding factors (Fig 1):

(1) The total number of individuals of that taxon present in the area.

(2) The taxon's activity patterns.

(3) Movement patterns of the taxon.

Observed trap catches are due to the joint effects of these three factors. The problem now arises: how to disentangle the three factors of detectability? To illustrate the complexity of the issue, consider a case where we survey two taxa – taxon A and taxon B. Let us assume that we catch a few individuals of taxon A, and many individuals of taxon B. This might lead us to believe that taxon B is more common in the area. However, we know that taxon A moves over greater areas and only tends to be active during warm, sunny days, while taxon B moves over small areas and is active independent of weather conditions. If our sampling period was during cloudy, cold days, we could then infer that the most common taxa in the traps does not necessarily equal the most common taxa in the area. We also know that taxon A has a larger area of movement, meaning it might be more regionally common than the locally abundant taxon B. The seemingly straightforward conclusions drawn from a sample has suddenly become a complex issue.

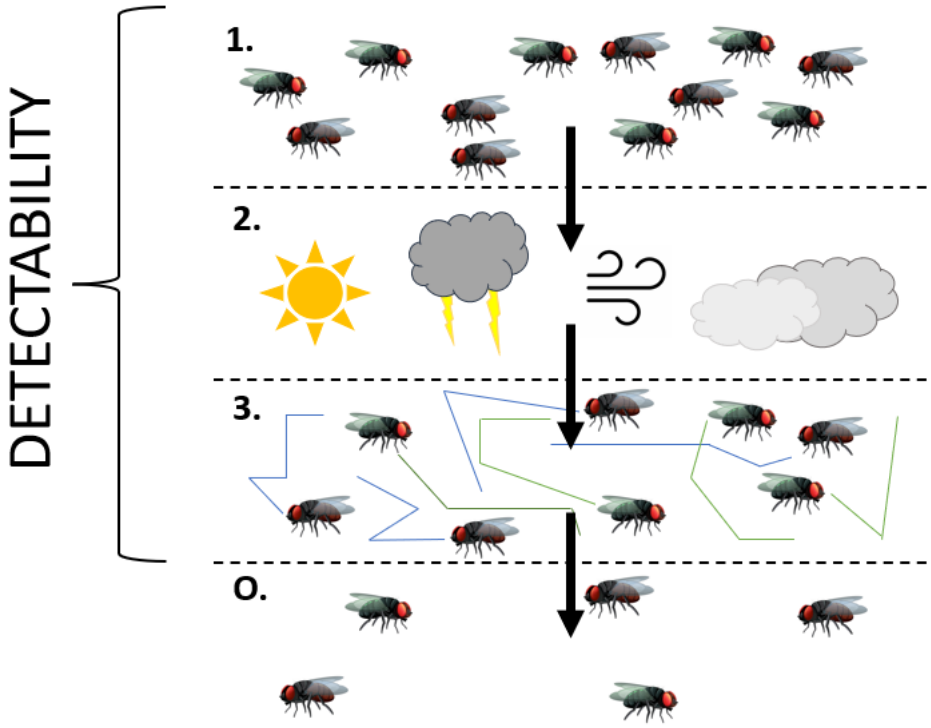

Figure 1. The three factors influencing detectability of insects: 1. The total number of individuals present in the area. 2. Abiotic factors affecting the insects' activity. 3. The movement pattern of the insects. The total number of individuals one could sample is equal to the total population sizes in the area. Weather might, however, cause only a subset of those to be active during the sampling period. The movement patterns of those active insects will then decide how likely they are to encounter a trap. After the total population has been subjected to each of these factors of detectability, the observed trap catch (O.) remains.

The importance of resolving the relative impact of the different factors 1-3 (above) is further revealed by the following example. Consider a case where samples from a Malaise trap is to form the groundwork for conservation measurements (Such as what area to protect) or a red-list assessment (As based on estimates of population sizes or ranges). Acquiring the knowledge of how species' movement patterns and abiotic factors influence the species' detectability may then drastically change conclusions drawn from this sampling effort, since the abundance in the trap will depend only partially on the actual abundances of the species in the area. Knowledge of the species' detectability would thus be essential for assessing surrounding insect community structures or to make effective conservation-based decisions.

However, acquiring knowledge of each of these factors for a multitude of species is logically impossible. As a more practical solution, we need general proxies to substitute species-level detectability. Here, the idea that some measurable species characteristics, known as *traits*, may offer reliable indicators of species-level properties has recently percolated ecology (Davis *et al.* 2016, Noriega *et al.* 2018). These could include body length, wing: body ratio, or weight, to mention a few. Another approach is to decrease resolution, using a broader taxonomic level as baseline. This way, while species identities may change between locations, the most common genera or families sampled will remain the same (Fraser *et al.*

2007, Fraser *et al.* 2008). Finding the general detectability of traits or taxonomic families could thus be an effective way of assessing true insect densities across many different habitats and regions in a standardized manner.

The current activity of a taxon (at any sufficient taxonomic level), or any trait level, will depend on environmental factors. In effect, the activity of any insect will depend on that insects' reaction to the current weather conditions. Earlier studies have identified temperature and daily rhythm as two main factors affecting activity of insects (Williams 1940, Briers *et al.* 2003, Genoud *et al.* 2021). Other factors that might be of importance are wind speed, exposure to sun radiation, and relative humidity (Williams 1940, Herrera 1990, Peng *et al.* 1992).

To summarize, trap catches from Malaise traps depend on the three factors of detectability: the number of individuals present in the area, their movement patterns, and their activity patterns. To acquire knowledge of these factors for every species is logistically impossible. Rather, we can aim for a broader perspective to understand more general patterns by studying the detectability of higher taxonomic units, or by using trait proxies.

Observational models

If we knew the effect of weather conditions on activity of a certain taxon, together with the odds of that taxon ending up in a trap whilst active (i.e., its' movement patterns), we could estimate the true densities of that taxon in the area by employing an observational model. The model is based on the number of individuals sampled (S) for each taxonomic identity or trait value (T). We call this parameter (S_T). As stated, the observed number of individuals is a function of the three factors of detectability. We here denote them as α for true densities, β for movement pattern, and γ for activity. The observed number of individuals of taxon T is thus a function of α, β, and γ:

$$S_T(\alpha_T, \beta_T, \gamma_T)$$

Activity, γ_T, will be dependent on the sum of positive and negative effects from all examined environmental variables (Williams 1940). As such, γT could be rewritten as :

$\gamma_T = \gamma_{Temp}$ (activity due to temperature) + γ_{Wind} (activity due to wind) + γ_{RH} (activity due to relative humidity). However, for simplicity we keep it as γ_T here.

We can simplify the model by adding β_T and γ_T, movement and activity, together to constitute taxon T's detectability constant: $T_x(\beta_T, \gamma_T)$. True density of taxon T multiplied with T_x thus provides observed trap catch density. Now, the last factor to add is time-dependence. We assume that the loss of insects in the area due to sampling is balanced by continuous birth and immigration. We also assume that the effect of weather on insect catches is balanced over time, so that with increased sampling time the number of insects caught per day approaches a general value. This leads us to say that the number of insects caught in a malaise trap over time should increase linearly by each trapping day. We can express this as y_T=k * d, where d

is the amounts of days of trapping, k is the mean number of insects caught per day and Y_T the number of individuals with trait or taxonomic identity T that have been sampled during the period. Since at day zero there are no insects caught, we exclude an intercept.

By dividing our previous formula by this expression, we thus get an estimate of insect abundance in the area per day over the study period. This is a useful method of scaling results, as comparing insect densities between different areas would otherwise demand equal days of sampling. We can now write our final formula as:

$$\alpha_T = S_T * T_x(\beta_T, \gamma_T) / (k * d)$$

Now, for all taxa or traits observed in a trap, we would ideally have a T_x. We could then calculate the true densities of insects in the area by the formula:

$$\alpha_{Total} = (S_{T1} * T_1(\beta_{T1}, \gamma_{T1}) + S_{T2} * T_2(\beta_{T2}, \gamma_{T2}) \ \ldots + S_{Tx} * T_x(\beta_{Tx}, \gamma_{Tx})) / (k * d)$$

However, acquiring the specific T_x for each taxon could come close to, or even be, impossible. Even if we were to have the exact effect of activity and movement on detectability, factors such as presence of predators, food availability and diseases may also play a role in determining insect behavior and subsequent densities (Bell 2003, Knell & Webberley 2004, Malmqvist *et al.* 2018). A more realistic option would be to gain knowledge about how movement and activity, through environmental factors, influence taxa or traits and to what extent. In effect, we would ask "do we expect the true densities to be much larger or around equal to our catches based on the environment and taxon studied?", rather than "based on this catch, environment and taxon, what are the exact densities in the area?". The observational model described still explains holds true for this line of thought, although we do not employ it for exact measurements of population sizes.

Studies evaluating the effect of weather on insect activity across a temporal scale of days or weeks (Williams 1961, Taylor 1963, Briers *et al.* 2003) are important to understand broader environmental impacts on abundances. However, as stated by Taylor (1963), you need to evaluate the daily rhythm of the species coupled with small fluctuations in weather to understand the exact effects of these factors on activity. Knowing the daily rhythm of an insect will allow one to specify the hours of the day at which weather will influence activity. In effect, while Malaise traps might be emptied first after several days, only a few hours of each day will have contributed to most of the sampled insect from each group. The weather differences during these hours may be much less prominent than overall differences throughout the days and thus obscure true activity patterns in relation to weather. It is then crucial to sample at a temporal scale where the effects of weather during only these hours of the insects' activity can be assessed (Taylor 1963).

In conclusion, if we were to know the influence of the detectability factors on trap catches of insects with different trait values for a given trait, or on insects of different taxonomic affinity, we could create an observational model that adjusted for that detectability. While acquiring raw numbers is a very complex matter, a general understanding of different

environmental factors' influence on activity of different insect groups, in combination with the group's movement patterns, could provide a sufficient metric of detectability.

Aims of the study

This study aimed to investigate mainly how activity (through environmental factors) influence detectability of insects commonly found in Malaise traps. My objective was to contribute to a better understanding of Malaise trap samples in relation to surrounding insect densities, and to evaluate the robustness of Malaise trap samples across weather conditions. I also aimed to develop a method by which data on movement patterns of insects around Malaise traps could be collected. By establishing the relative detectability of insects in relation to taxonomic identity and traits, one can account for not only trap catches but also the amounts of individuals moving within or through the area while avoiding the trap. In short, I aimed to find an approximation of T_x in the formula established above. However, the process of developing diffusion models to gain insights regarding movement patterns of insects was beyond the scope of this study. Thus, the aim here regarding movement patterns was instead to develop a suitable method for obtaining data on movement patterns of insects in relation to Malaise traps. Future studies could then use this method to obtain data for such diffusion models.

To infer how the environment affects the activity of insects and thus detectability in Malaise traps, I sampled Malaise traps at short intervals over a period exhibiting different weather conditions. I divided the insects into different taxonomic groups, measured physical trait values, and compared the catches for each group or trait value with fluctuations in temperature, wind speed, cloud cover, rain amounts, and relative humidity. To pave way for studies examining movement patterns of different insects in relation to Malaise traps in the future, I performed a mark-recapture experiment with insects commonly caught in Malaise traps. The use of mark-recapture data in understanding insect dispersal has been greatly facilitated by Bayesian diffusion models (Ovaskainen et al. 2008a, Ovaskainen et al. 2008b). This approach allows the quantification of dispersal in heterogeneous environments based on data of where and when the marked insects were recaptured. Recently, this approach was further developed to include species traits in joint species movement models (JSMM), allowing us to determine how species traits affect movement patterns and whether phylogenetically similar species share similar movement patterns (Ovaskainen et al. 2019). It is thus fair to say that there exist viable methods for analyzing data from mark-recapture experiments of insect movement patterns, would such data be available.

Since most species caught in Malaise traps are small and fragile, I had to develop a novel marking technique for the mark-recapture experiment. Methods for bulk-marking small and fragile species such as flies, mosquitoes, and parasitic wasps, have been developed since the 1920's (Geiger *et al.* 1919) and have in many cases involved fluorescent dyes and dusts to mark the insects. (Including Stern & Mueller 1968, Dickens & Brant 2014, Culbert *et al.* 2020). However, these studies have exclusively used single species or lab-reared insects, in

contrast to wild, multi-species catches for which I needed to develop a marking technique. In addition, no study concerning marking of these kind of insects had so far been conducted under field conditions (Dickens and Brant 2014). Some have pointed to the need for such a study as there are many factors which might affect marked insects in their natural environment, not discovered in a lab setting (Dickens & Brant 2014; Culbert et al 2020). This study thus aimed to develop a marking method involving fluorescent dusts that could be used to mark many different orders of wild-caught insects at once without having a significant impact on the insects' lifespan or flight ability. Development of such a method could be useful in a multitude of scientific fields, including pest control of agricultural lands, tracking species movements in natural environments, and following host choices of parasitic wasps.

To summarize, this study aimed to provide unique insights into how abiotic factors affect insect's detectability in Malaise traps, and if mark-recapture is a viable method for future endeavors surrounding insect movement patterns in relation to Malaise traps. To perform the mark-recapture experiment, a method for bulk marking wild insects with fluorescent dust was developed. To examine abiotic factors' effect on taxon and trait detectability, I sampled Malaise traps during different weather conditions with short time intervals. Altogether, this study provided crucial knowledge for anyone interested in understanding insect's activity patterns, or for people using Malaise traps in their scientific research.

Methods

Overview

To infer what the catches of Malaise traps can reveal concerning surrounding insect communities, I strived for a comprehensive approach. As the relative detectability of a species is determined partly by its' movement patterns, I aimed to develop a mark-recapture experiment that would allow for future studies of insect movement patterns in relation to Malaise traps. To achieve this aim, I had to develop a method for the mass-marking of insects. To validate the method, I in turn had to ensure that the markings stayed on in the natural environment, and preferably during storage in ethanol. At the same time, the markings should not hamper the insects' flight ability or general survival. Furthermore, as the relative detectability of a species is also determined partly by its activity, I strived to infer how weather might affect insect activity. In effect, I needed to examine how the catches of different taxa and traits changed with weather conditions. To address these aspects of detectability, I took a 3-step approach.

1) To ensure that the mass-marking of insects did not affect their behavior, I conducted small-scale survival and flight ability experiments prior to the mark-recapture study. To obtain data on survival and flight ability after marking from different species and taxonomic groups, I mass-reared different species of Diptera to use for the experiments and coupled this with other mass-reared insects ordered from a horticultural company.

2) To mark large amounts of wild insects quickly, I developed a method of marking these insects using plastic boxes. These boxes replaced the collecting heads of the traps and thus both caught and marked insects, which could later be transported to the mark-recapture area in the boxes for release.

3) To infer how the insects' activity might change with weather conditions, I emptied Malaise traps every second hour over a period of five days. This provided High-Resolution detectability data, which I used to compare changes in catches over time with changes in weather conditions, and thereby distinguish what factors affect the activity patterns of insect communities over time, and how.

The steps are illustrated in schematic figure 2 and described in detail below.

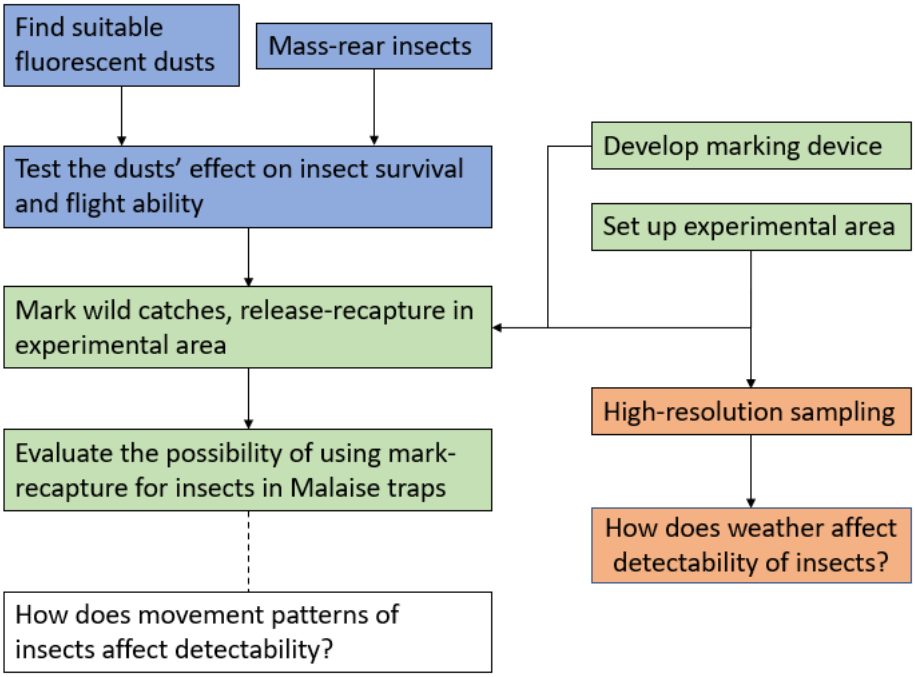

Figure 2. Schematic figure of the workflow during the study. The colors illustrate the three steps described above. Blue = step 1, green = step 2, orange = step 3. To understand how different taxa and traits affect detectability in Malaise traps I conducted several experiments. First, to develop a method for conducting mark-recapture studies of wild insects, I found suitable fluorescent dusts and mass-reared different insect species. To make sure that the fluorescent dust did not affect insect behavior, I then conducted small-scale survival and flight experiments. At the same time, I also set up the Malaise traps in the experimental area and developed a device for marking wild insects quickly. I then proceeded to release marked individuals as a mark-recapture trial and emptied the Malaise traps daily, while also conducting High-resolution sampling for five days during this period. The High-resolution data was used to investigate how weather conditions affect detectability of insects in Malaise traps. The developed mark-recapture technique for insects in Malaise traps can be used to infer how movement patterns of insects affect detectability.

Ex situ experiments

Mass rearing

To obtain insects for my small-scale survival and flight ability experiments, I mass-reared *Drosophila melanogaster* (Diptera: Drosophilidae)*, Drosophila simulans* (Diptera: Drosophilidae)*,* and *Drosophila littoralis* (Diptera: Drosophilidae) in climate chambers. I put the insects in vials filled with a nutrition medium in which imagos could lay eggs and larvae could feed. I switched the vials on a weekly basis. To match natural conditions, I set the climate chambers at 24h light, 60% humidity and 19℃. All procedures concerning my mass-rearing of flies followed the protocol used at University of Jyväskylä (Kankare, M. pers. com). To increase the taxonomic span of insects used, I also obtained adult individuals of *Sphaerophoria ruepellii* (Diptera: Syrphidae)*, Episyrphus balteatus* (Diptera: Syrphidae), *Orius majusculus* (Hemiptera: Anthocoridae*), Macrolophus pygmaeus* (Hemiptera: Miridae)*,* and *Diglyphus isaea* (Hymenoptera: Eulophidae) from the company *BiTaxons* (Forssa, Finland)*.*

Survival

To infer whether dusting insects with fluorescent dusts might affect their longevity, I marked four species of flies; *Drosophila melanogaster, D. littoralis, D. simulans* and *Episyrphus balteatus*, two species of Hemiptera; *Orius majusculus and Macrolophus pygmaeus* and one species of Hymenoptera, *Diglyphus isaea*, with the fluorescent dusts and put the insects in containers to evaluate post-marking survival. I used a yellow dust (Product ID. 35898-2) from *Partykungen* (Gävle, Sweden) as well as one red dust (Product ID. TP-45) and one blue dust (Product ID. TP-49) from *Radiant Color* (Houthalen, Belgium) for all experiments.

To ease the handling process, I cooled the insects in a fridge for 15-30 minutes before marking. I then transferred them to small plastic containers, container each dusted with one of the fluorescent colors. I added 5-20 individuals per container, depending on the size of the species. Each plastic container was later treated as one sample. To ensure that all individuals would be readily marked I used 20 mg of the low affinity yellow dust from *Partykungen*, compared to only 8 mg of the *Radiant color* dusts. To ensure all insects were equally marked, I gently rotated the colored containers with the insects inside for 30 seconds; a technique developed by Culbert et al. (2020). I did this for three containers per color and species, plus three uncolored control containers for each species, amounting to 12 containers per species in total. To allow the insects to clean themselves after marking, I then transferred them to clean containers where they were left undisturbed for one hour. To make sure that the control groups experienced the same methodology as the marked individuals, insects from the control containers were also moved to new containers.

After one hour, I transferred the samples to bigger plastic cups. To increase moisture in the cups without soaking the insects, I filled the cups with 70 ml of water and added a piece of rubber foam (thickness 3 cm) on the bottom. I made sure that the water did not reach above the foam before adding the insects. To provide an energy source for the insects, I placed a cotton ball dipped in a sugar-water mix on top of the foam. I then added the marked insects, covered the top of the cups with a fine mesh and placed them in a climate chamber. To mimic

a typical Swedish environment, I set the climate chamber on 20° Celsius at 60% humidity and with a 16-8 light to darkness ratio.

I monitored the insects daily for the first 5 days and recorded the number of individuals alive at each day. If the survival continued to change daily, I continued to monitor them daily. If, however the survival remained unchanged for more than 3 days, I switched to monitoring the insects every second day. I continued the experiments until there were no more alive individuals, or until a maximum limit of 17 days had been reached.

To make sure all individuals remained visibly marked after death, I examined them under a UV-torchlight with a wavelength of 395-400 nm and a luminosity of 0.08-0.15 cd (Perel EFL41UV) in complete darkness. This torchlight was used in all subsequent experiments mentioning UV-light.

Flight-ability

To evaluate how the dusting of insects might affect their flight-ability, I marked the species *Sphaerophoria ruepellii, D. melanogaster, D. littoralis, D. simulans, M. pygmaeus* and *D. isaea* following the same procedure as in the survival experiments. After marking and subsequent self-cleaning, I released the insect in mesh mesocosms of 56x70x36 cm (Fig 3). Three species were released at a time in the same mesocosm with all colors and control groups included in each mesocosm. Along a parallel in the mesocosms, I hung a line of 5 yellow sticky traps covering the entire middle area of the enclosure (Fig 3). I put the mesocosms outdoors for 48 hours and protected them with a roof if rain was prominent. Afterwards I collected the sticky traps and examined the number of individuals caught in the traps, along with their marking colors, under UV light. I could then compare the catches of unmarked and marked individuals and conclude whether the fluorescent dusts hampered the insect's capability of flight. If more insects from the control groups would be caught in the sticky traps compared to marked individuals, this would suggest that the dusts do hamper the insects' flight capability. To obtain more data on recapture rates, I repeated the experiment twice.

Figure 3. The mesh mesocosms, used for flight-experiments. I marked 6 species of insects with red, blue, or yellow fluorescent dust and released them together with unmarked control groups for all species. I hung five yellow sticky traps across the mesocosm to catch insects capable of flying. By this method, I could conclude whether the fluorescent dusts hampered their capability of flight. If more insects from the control groups were caught in the sticky traps compared to marked individuals, this would suggest that the dusts do hamper their flight capability.

In situ experiments

Live catching and marking

To catch insects alive in Malaise traps for subsequent marking, I designed a novel trapping device from plastic boxes of 11x17x17 cm (Fig 4; 5). I cut a Malaise bottle in half and glued the top part at a hole of the lid of a plastic box. To avoiding insects being crushed against the lid floor when transported, I attached a foam-bottom around the rest of the lid with glue. To increase air circulation and thus keep the temperatures from reaching excessive levels inside the box, I cut a hole on each side, as well as on the top, of approximately 3-4x5-6 cm, and covered these holes with a fine mesh. In another attempt to increase air circulation, I covered one of the mesh-holes with a 50x50mm computer fan on some of the boxes. I connected the fans to a battery holder glued onto the outside of the box. The fan would create a wind funnel, decreasing temperature within the box on warm days. The decrease in temperature would not only promote survival but also help calm the insects (Álvarez *et al.* 2015).

To achieve efficient self-marking of insects, I painted the walls and roof of the box with a thin layer of fluorescent power using a small brush. This made it possible for the insects to mark themselves passively by walking or flying into the sides or roof of the traps. I aimed with this method to increase their post-marking survival. The idea followed the logic that insects would by walking or flying attach the marking medium to non-lethal part on their

bodies such as legs, abdomens, or scutella, rather than having eyes, antennae or spiracles covered. To provide shade and hiding spots for the insects, I added pieces of egg cartons inside the boxes before attaching the lids.

I attached the boxes upside down on the Malaise traps using the glued-on lids (Fig 5). Note that this is not possible for all models of Malaise traps, as they need to have double attachment points. The traps used in this study were ez-Malaise Trap II, Townes Style (Product ID. BT1012) from MegaView Science Co., Ltd (Taichung, Taiwan).

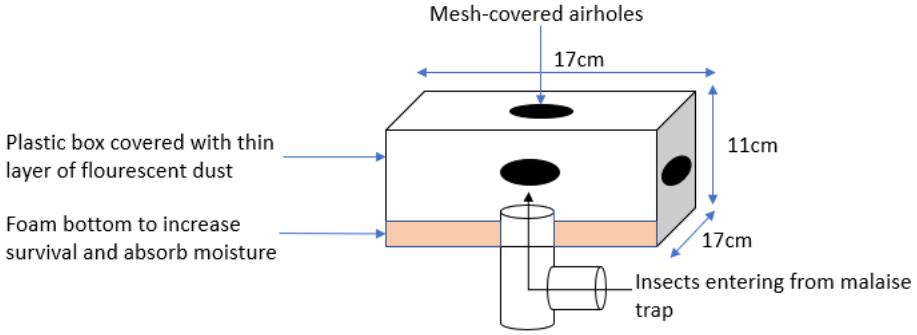

Figure 4. A novel trapping device for catching insects alive in Malaise traps. Upon capture, insects tend to move upwards within the Malaise trap and then end up in a plastic box. The bottom of the box has a sponge layer with pieces of egg cartons on top for shade and shelter. A mesh covered hole can be found in all directions except the bottom to increase air movement.

Figure 5 The novel trapping device in field. In the left picture, the trap has been painted with yellow dust and carries no fan. Notice the upside-down Malaise bottles glued to the lids of the boxes, attached to the Malaise traps. In the picture to the right, the inside has been painted with red fluorescent dust and a battery-driven fan is attached to one of the mesh-covered air holes.

I attached the boxes onto the Malaise traps between 8:00 and 9:00 in the morning and collected them 24 hours later. I transferred the boxes to the experimental areas and, using a funnel, poured the insects from the boxes into plastic bags. To avoid crushing the insects, I attached cardboard to the inner sides of the plastic bags, keeping the bags three-dimensional (Fig 6). To be able to later identify the taxonomic identity and size of insects released from each box, I took several pictures from above of the insects within the bags with millimeter-paper underneath. From the plastic bags, I poured the insects onto open petri dishes. To avoid the insect quickly fleeing the area, providing them time to calm down and adapt a more natural movement behavior before leaving, I took two precautions. Firstly, I placed buckets upside-down on top of each petri dish. I replaced the bottoms of these buckets with metal meshes and covered the mesh with hay. This provided a shaded, protected environment for the insects and forced them to climb through the hay before escaping. Secondly, I erected a tarpaulin 2 meters above the buckets to avoid insects flying straight up and disappearing from the area once they emerged through the hay (Fig 7). The tarpaulin also protected the emerging insects from potential rain and provided the buckets with shade.

To be able to exclude individuals that died on the petri dish from the analyses, I removed the petri dishes 24 hours after release and recorded the remaining insects by pictures.

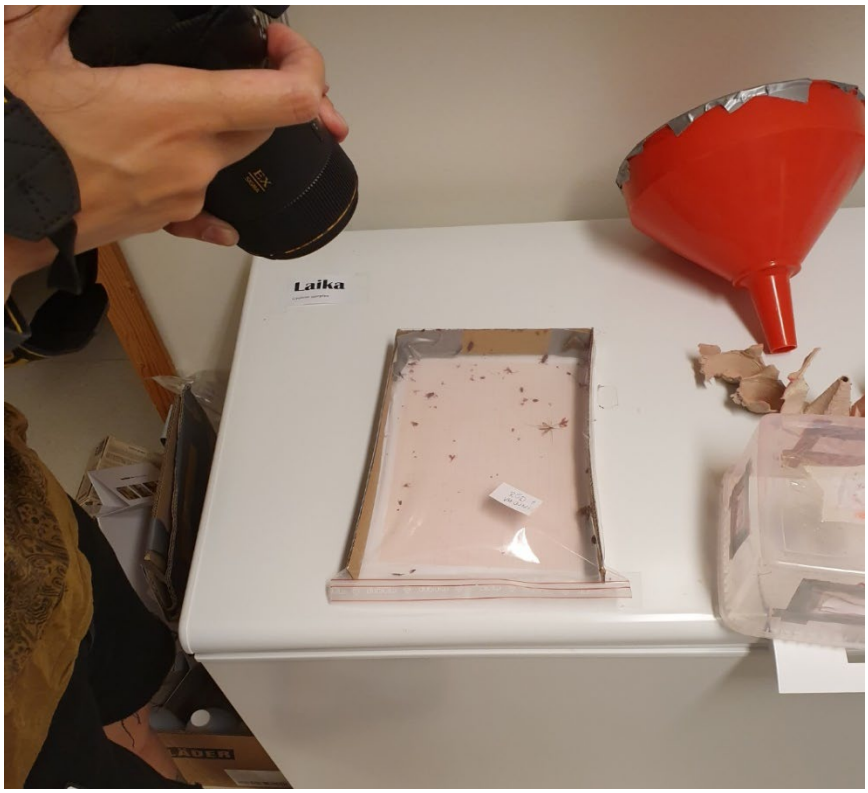

Figure 6. A lab trial of my method of pouring the insects from the boxes into plastic bags using a funnel. A colored box can be seen to the right and above it the funnel. I photographed the insects inside the bags with a millimeter-paper behind to record size and transferred them to petri dishes for release afterwards.

Figure 7. The experimental area. To avoid the insects escaping upwards after release, I erected a tarpaulin above the blue buckets from which the insects emerged. The tarpaulin also protected the emerging insects from potential rain and provided the buckets with shade.

Recapture

To obtain a natural composition of species for the mark-recapture trials, I set up 16 Malaise traps around the premise of The Swedish University of Agricultural Sciences in Uppsala to sample from. I attached the marked boxes between 8:00 and 9:30 in the morning and collected them after 24 hours, attaching new boxes at the same time for further collection the following day.

As a trial I used two experimental areas consisting of 16 Malaise traps each, set up in a square network with 10 meters between each trap (Fig 8). The first site is henceforth referred to as SEL (Selknä Experimental Area, N59°51'25.646", E17°53'26.933"), and the second site VAT (Vattholma Experimental Area, N60°01'29.478", E17°45'4.809"). Site SEL consisted of mainly spruce (*Picea abies*) and pine (*Pinus sylvestris)* of different ages and the understory of younger versions of the same species. The forest floor was dominated by ferns (*Polypodiopsida*) and blueberries (*Vaccinium myrtillus*). A gravel road passed about 20 meters east of the site. Site VAT was dominated by 40–60-year-old pines. The understory was also represented by pine, with scattered findings of spruce and thorny bushes. The forest floor was dominated by wild strawberries (*Fragaria vesca*), horsetails (*Equisetaceae*), and graminoids. A tarmac road passed about 20 meters south of the site.

In each area I released insects marked with one color of fluorescent dust (blue, red, yellow) every second day. That is, day one I released a blue batch in SEL, day two a blue batch in VAT, day three a red batch in SEL, day four a red batch in VAT, and so on. Before each new release I emptied the Malaise bottles at the present site. The Malaise bottles I used were 500ml volume Wide-Mouth LDPE Bottles from Thermo Scientific (Rochester, USA), filled to a quarter with 70% ethanol.

To limit the risk of mixing findings of individuals of the same color released on different occasions at the same site, I continued to empty the Malaise traps every second day for 5 days after insects of all colors had been released. I then repeated the experiment following the same procedure. To increase the number of released individuals, I released mass-reared insects during one occasion.

To count marked individuals, I poured the content of each Malaise bottle onto a tray and scanned the insects under UV-light with a microscope. Each marked individual was identified to a minimum taxonomic level of Order. To account for differences in life history and physical build within Orders, I split the found individuals of Hymenoptera into *Symphyta, Aculeata* and *Parasitica*. Using the same logic, Dipterans were divided into *Brachycera* and *Nematocera*. I also split *Lepidoptera* into *Papilionoidea* (here all butterflies) and *Lepidoptera* (All other lepidopterans), due to the difference in wing size and diurnal patterns between the two groups. To infer how many days the insects had been active in the area before recapture, I also noted marked color.

To make sure that the fluorescent color would be visible after the insects had been submerged in ethanol, I also conducted trials with wild individuals caught in the marking-boxes, which I poured directly through a funnel into bottles with ethanol. I mixed insects marked with all three colors, as well as a known number of unmarked individuals, in the ethanol-filled bottles and left them for 72 hours. After this time, I screened the samples using the technique mentioned above. I repeated this test with four different Malaise bottles. All marked individuals remained visible and only once did one unmarked individual become secondary marked inside the bottles.

To obtain further insects for release, I moved eight traps from site 1 to the proximity of site 2 and attached collecting boxes on top. To obtain diffusion rate data from a larger area rather than from two lesser areas, I moved the other eight traps from site 1 to site 2. To further increase the experimental area, I changed the experimental design slightly – around the release point I set up a square of eight traps of which the horizontal and vertical traps were five meters from the release point and the diagonal traps thus were set seven meters from the release point. I then set up another square of eight traps so that each horizontal and vertical trap were ten meters from the first square traps (14 meters for diagonal traps), and a final square of traps 15 meters from the second square traps (23 meters for diagonal traps) (Fig 9). I proceeded to use this design for the remaining 4 weeks during which I released 13 daily catches of marked individuals, between the 26th of June to the 21st of July. On one occasion, I also released mass-reared insects. After insects of all three colors had been released in consecutive order, I emptied the traps for 2-4 days without adding additional individuals.

The analysis process for finding marked individuals did not differ from the one described in the trial experiments.

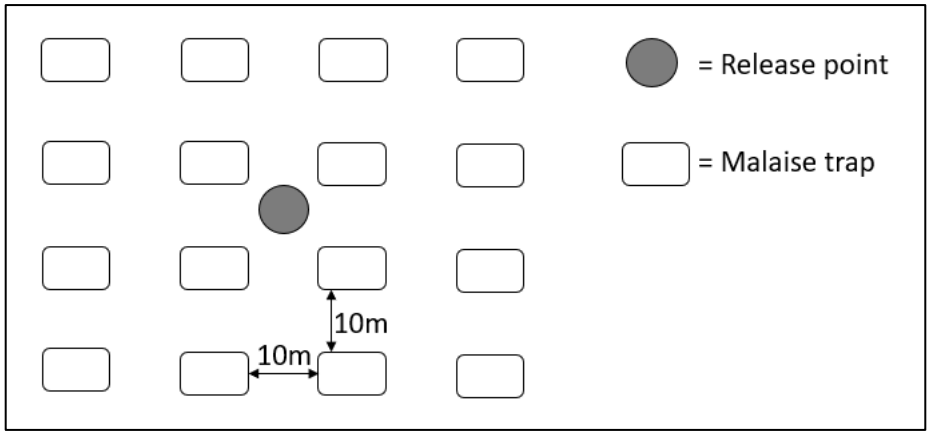

Figure 8. The experimental design for the trial experiments. 16 Malaise traps were set up in an even network of 4x4 traps 10 meters apart around the release point. This design was used simultaneously at two different locations.

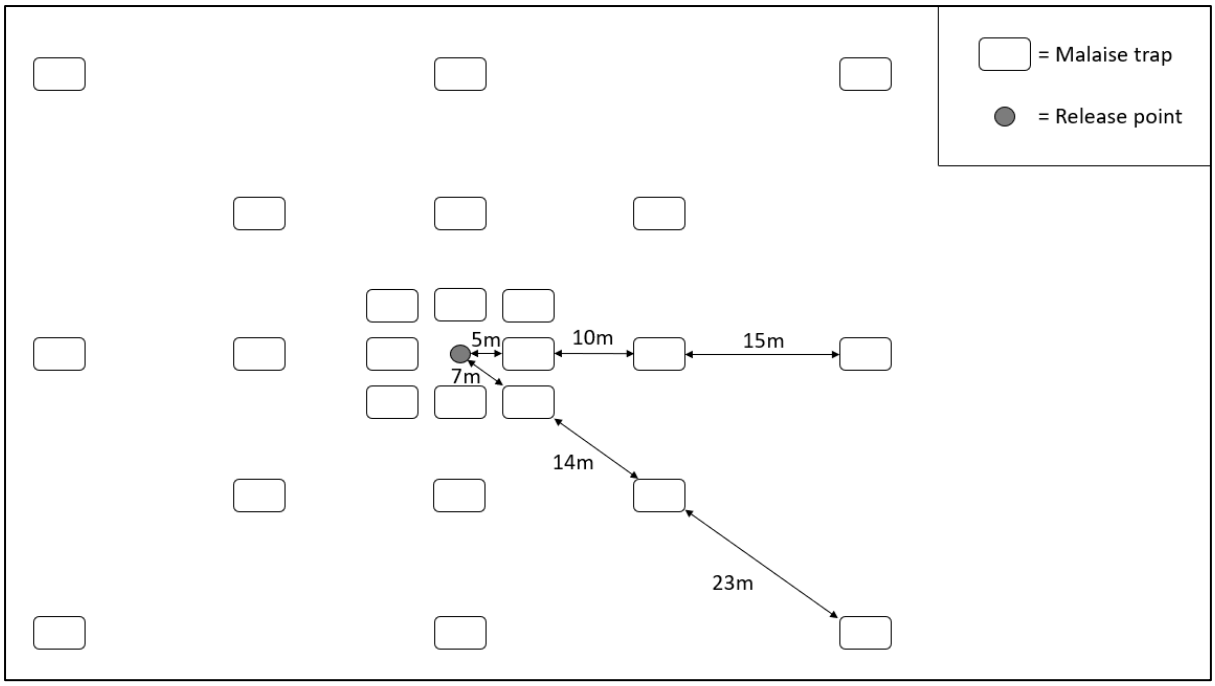

Figure 9. The experimental design, used to gather the analyzed data, employed at site VAT. 24 Malaise traps were set up divided in three squares around the insect release point. The distance from the release point to the first vertical and horizontal traps were 5 meters, from first to second square vertical and horizontal traps 10 meters and the distance between the second and final square vertical and horizontal traps 15 meters. This caused the diagonal set traps to be between release point and first trap 7 meters, between first and second square traps 14 meters and between second and final square traps 23 meters. The whole area encompassed 360 square meters.

High-Resolution sampling

To infer how weather might affect the insects' temporal detectability, I emptied the 24 Malaise traps at site VAT used in the experimental design seen in Fig. 5 with short intervals. This part of the study is henceforth referred to as "High-Resolution Sampling". To be able to connect temporal changes in species activity with weather conditions, starting at 16:00 on the 15th of July and ending on the 20th at 16:00, I emptied the Malaise traps every second hour between 06:00-22:00. Furthermore, for each sampling occasion I recorded temperature, relative humidity, wind speed, wind direction, and possible rain amounts from a nearby weather station, as well as cloud cover by observation. To record species activity patterns during night, I also emptied the traps at 02:00. To establish whether different groups of insects respond differently to weather conditions, I identified the caught specimens to a taxonomic level of family for all Dipterans and Hymenopterans except the superfamily *Chalcidoidea* within *Hymenoptera*. This group was only identified to superfamily level due to difficulties in assigning a lower taxonomic level without risking misidentification. Furthermore, to speed up the identification task all insects not belonging to *Diptera, Hymenoptera, Coleoptera, Lepidoptera* and *Hemiptera* were identified to taxonomic level of Order. This was seen as reasonable due to the low number of individuals usually found in Malaise traps of other Orders than those mentioned (Matthew & Matthew 2017). In addition, to simplify identification of a large group with similar morphology, all microlepidopteras were grouped as such with no further identification. Hereafter, *taxon* is used to denote the lowest taxonomic level used.

To be able to connect activity patterns with physical traits, I measured wing area and body area for a subset of individuals from each taxon. Knowledge of abundance of different taxa throughout time, coupled with their traits, allowed me to examine not only whether detectability changes with weather but also how it changes between taxa. Furthermore, I could investigate how different physical traits affected detectability in relation to weather (Fig 10).

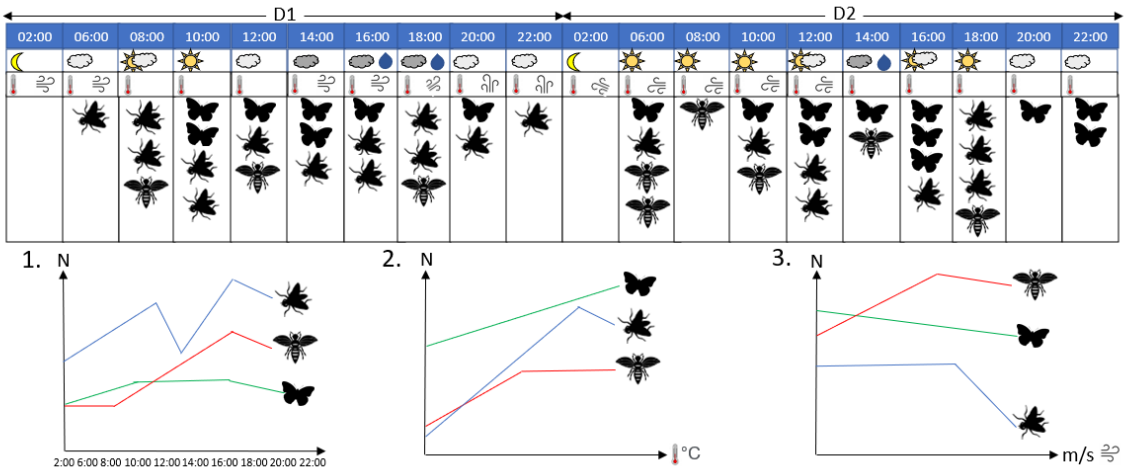

Figure 10. Schematic figures of how taxon-specific prevalence in trap catches can be used together with information of abiotic conditions to get a direct estimate of 1. Time specific detectability of different taxon or traits, 2. Temperature specific detectability, 3. Wind-speed specific detectability. As shown in the figure, traps were emptied every second hour between 06:00 and 22:00, with an additional emptying at 02:00. Wind-speed and wind direction, weather, temperature, and possible rain amounts were recorded with each emptying. This procedure is referred to as "High-Resolution Sampling". The values in the figure are fabricated and do not represent real data.

Data analysis

I compiled data using Microsoft Excel (2018) and performed all analyses in R studio using R 4.2.3 (R Posit Team 2023; R Core Team 2023).

Survival experiment

To evaluate whether the different color treatments had a negative effect on survival of the mass-reared insects, I built a generalized linear mixed model using package 'glmmTMB' in R (Brooks *et al.* 2017). I used death ratio as response variable (applying a "dead/not dead" approach), and species, day, and treatment as fixed effect factors, along with the three-way interaction between them (Table 2.1). Furthermore, to account for the fact that each experiment was conducted three times, I added the experimental round as a nested random factor within each treatment.

D. melanogaster exhibited complete separation of data for two treatments (i.e., no overlap in dates between observations of survival and death; Fig 11). To facilitate model fit , the species was therefore removed from further analysis.

To detect overdispersion, I used the package "performance" in R (Lüdecke *et al.* 2021). As my data was indeed overdispersed, I addressed the issue by adding a random factor of row ID to the model. To visually assess whether all other assumptions of a GLMM were met, I used the package 'DHARMa' in R (Hartig 2022). To examine the significance of the different explanatory variables and their interactions for the response, I used the 'car' package in R (Fox & Weisberg 2019) to conduct a type III Wald chi-square test on the model specified.

Flight ability

To evaluate whether the flight ability of the different insects used was negatively affected by color application, I ran a generalized linear mixed model with a binomial distribution and logit link. As response variable, I used amount of recatches per species (applying a "recaught/not recaught" approach), with the different species and the three UV-color treatments (including an uncolored control group), as predictors (Table 2.2). To establish whether different species responded differently to the treatments, I also added the interaction between species and treatment. As I detected an overdispersion of errors (residual deviance = 77.984 on 28 degrees of freedom), I changed the model to apply a quasi-binomial distribution. I assessed all other assumptions of a GLMM visually using the 'DHARMa' package in R. To examine the impact of the different explanatory variables and their interaction on the response, I conducted a type III Wald chi-square test on the model specified using the 'car' package in R.

High-Resolution Sampling

Environmental models

Altogether, the Malaise traps caught 5511 individuals belonging to 88 taxa during the sampling period (Fig 11). To evaluate whether wing area and body area of the insects might explain their activity patterns in relation to environmental variables, as may be expected if smaller insects are more active at higher temperatures (Willmer & Unwin 1981, Herrera 1990), I measured these trait values for 1554 individuals from 87 taxa. From these measurements, I excluded one family of Coleoptera due to difficulties in obtaining meaningful measurements of wing area.

During the sampling period, temperatures varied between 9.8-21.3 degrees °C, usually peaking between 12:00-20:00, and reaching lowest values between 02:00-08:00. Wind speed varied between 0.5-4.7 m/s, cloud cover between 0-1 (0 corresponding no cloud cover, 1 meaning total cloud cover), relative humidity between 33.4-98.4%, and rain between 0-0.1mm. Since the amounts of precipitation were consistently small, I chose to remove this variable from any further analysis. Data on temperature, wind speed, and relative humidity were obtained from weather station *327 Björklinge*, provided by Trafikverket, with measurements every 30 minutes. This weather station is located approximately 10 kilometers east of the field site. To ensure that such a distance would not provide frauded data in comparison to the actual sampling site, I compared the data with data from another weather station, *326 Uppsala,* located approximately 15 kilometers south of the field site. Since the used variables were almost identical in values between these two sites, I deemed the field site, located almost in between them, to show insignificant fluctuations from measured data. To obtain average values of each variable during each 2h sampling period (or 4h sampling period during nights), I summed the values from each half hour measurement between last sampling to current sampling event and divided this by the number of half hour measurements.

To avoid zero inflation (i.e., having too many timesteps with zero findings) in the High-Resolution Sampling models, I excluded all taxa found in a total of less than 20 of the

timesteps or comprising less than 50 individuals. After this step, 4924 individuals from 17 taxa remained for further analysis (Table 1).

Table 1. A list of the 17 taxa that were found in 20 or more timesteps and comprised more than 50 individuals in total. Letter denotes order (D = Diptera, H = Hymenoptera, O = Other). Taxa scored at a level other than a taxonomic Family are denoted with a ''. Peak period tells time of day when the taxon peaked in abundance. UN = unknown, AF = afternoon, EV = evening, NI = night.*

Taxon	Peak period	Time steps present	Total amount
*Auchenhorryncha (O)**	UN	27	70
Braconidae (H)	AF-EV	25	58
Cecidomyiidae (D)	NI	50	2658
Ceraphronidae (H)	AF	28	89
*Chalcidoidea (H)**	AF	46	485
Diapridae (H)	AF	36	160
Dolichopodidae (D)	AF-EV	23	59
Hybotidae (D)	AF	22	65
Ichneumonidae (H)	AF	39	220
*Microlepidoptera (O)**	NI	37	167
Mycetophilidae (D)	AF-EV	35	95
Phoridae (D)	AF	35	136
Platygastridae (H)	AF	25	88
*Psocoptera (O)**	UN	30	70
Scelionidae (H)	AF	37	126
Sciaridae (D)	AF-EV	49	257
Throscidae (O)	NI	29	121

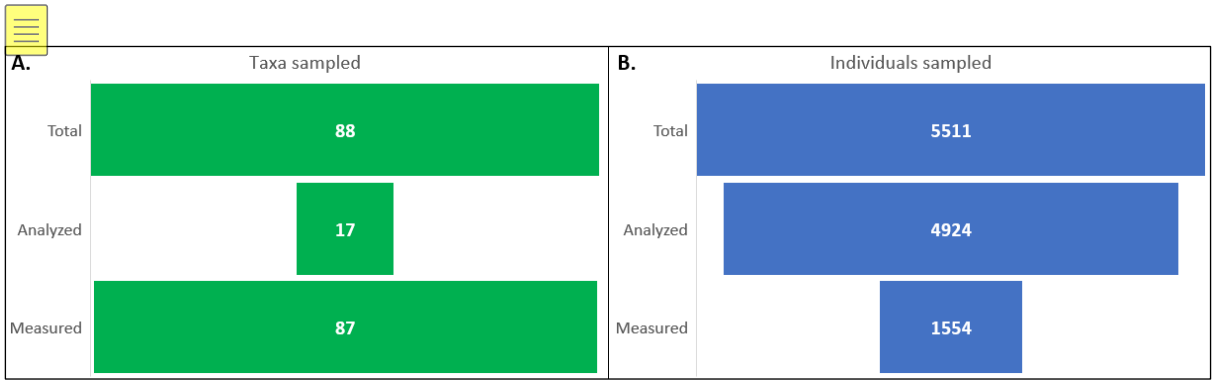

*Figure 11. **A**: Total number of **taxa** found during the sampling period, number of taxa that passed the analysis criteria, and number of taxa for which traits were measured. **B.** Total number of **individuals** found during the sampling period, number of individuals belonging to the 17 taxa that passed the analysis criteria (see Table 1), and total number of individuals for which traits (wing area and body area) were measured.*

To examine how abiotic factors affected the catches in the traps during the high-resolution sampling, I first scaled all continuous environmental variables to a mean of 0 and a standard deviation of 1 using the 'tidyverse' package in R (Wickham *et al.* 2019). To account for the fact that relative humidity and temperature were highly correlated (r = -0.675), I first fitted a linear model of relative humidity as a function of temperature and then used the residuals from the linear fit as a measure of the temperature-independent effect of relative humidity. I used a similar approach to the trait variables "Wing area" and "Body area", which were likewise correlated (r= 0.645). Here, I first log-transform both variables and then regressed wing area on body area. I then extracted the residuals from this regression as a measure of wing area independent of body area. All mentions hereafter of "wing area" or "relative humidity" will explicitly refer to these independent, residual effects.

As the circadian rhythm of insects has a major effect on their activity (Herrera 1990, Pawson *et al.* 2017, Genoud *et al.* 2021), I first created a model of observed insect abundances as a periodic function of time. To describe the time-abundance relationship, I created a sine–cosine function of time using the equation:

$$Fluctuation\ through\ time = \sin(2*\pi*Time\ of\ day/24) + \cos(2*\pi*Time\ of\ day/24)$$

To account for different taxa behaving differently throughout the day, I added an interaction effect between these time functions and taxon. Furthermore, to account for differences among the five days of sampling, I added date as a random factor to the model (Table 2.3).

To reduce the number of zeros in the dataset, I pooled the number of individuals caught across all Malaise traps at each timestep, per day and taxon. I then used the pooled amounts as the response variable in my Time Model (Fig 12). The model was first fitted assuming a Poisson distribution and log link using the R package 'glmmTMB'. However, since this model exhibited overdispersion of error, I switched to assuming a negative binomial distribution with a log link using the R package 'MASS' (Venables & Ripley 2002). Other model assumptions were visually assessed using the 'DHARMa' package in R.

To test whether environmental factors might influence the abundance of insects caught in the malaise traps, beyond the effect of time of day, I extracted the residuals from the Time Model discussed above (Fig 12A). These residuals represent the deviation from the expected daily rhythm for each taxon. However, to account for the fact that there will be hours of the day where certain taxa are inactive, regardless of environmental conditions (Taylor 1963), I removed datapoints at hours with consistently zero findings across all days for each taxon. These hours likely represent the taxa's inactive periods and should not be accounted for when comparing their activity with environmental conditions.

To establish how the environmental variables might affect abundance, I used the residuals from the Time Model as the response variable in a linear mixed model (Fig 12B). Since the residuals were based on counts, they were log-transformed. To account for the minimum residual value being -1, and to avoid log-transforming negative values and zeros, I added +2 to each residual value before log-transforming. As explanatory variables in this Environmental Model, I used all environmental variables (Cloud cover, Temperature, Relative humidity, and Wind speed), as well as their interaction with taxon. To account for sampling period, I again set date as a random factor.

I fitted the Environmental Model as a linear mixed effect model using R package 'lme4' (Bates *et al.* 2015) (Table 2.4). To examine the impact of the explanatory variables and their two-way interactions with taxon on the residual values, I conducted type III Wald chi-square tests on the model specified using the 'car' package in R. I then conducted model reduction based on the significance of the explanatory variables and their AIC scores. This allowed me to remove the interaction terms "cloud cover: taxon", "relative humidity: taxon", and "wind speed: taxon" (Table 2.5) from the final Environmental Model. I assessed the effects of the explanatory variables on the residuals from the Time Model with a type III ANOVA using the 'car' package in R. I checked model assumptions visually using the 'DHARMa' package in R.

Trait models

To test how physical traits might affect the activity of insects, and to separate between the effect of taxon identity and physical traits *per se*, I took two approaches. First, I aimed to establish whether taxa might exhibit different patterns of abundance in relation to environment due to size differences, specifically wing area and body area. If the taxa were to show such a pattern, this could partially explain any effect of the environment on abundance. E.g., some insects could then be more common at high wind speeds because of large physical size rather than taxonomic affinity.

To this aim, I used the R package 'emmeans' (Lengh 2023) to extract the temperature slope of each taxon from the Environmental Model. I used only the slopes from the temperature * taxon interaction since this was the only significant interaction in the Environmental Model. Furthermore, all other environmental variable * taxon interaction showed standard errors that exceeded the values of the slopes themselves, providing further evidence to exclude these from analysis. I then tested for an effect of the mean trait of the taxon-specific response (Fig 12C, Table 2.6). I did this by fitting a linear model, hereafter called the Taxon-Temperature Model, of the taxon-specific response of temperature as a function of mean body area and mean wing area.

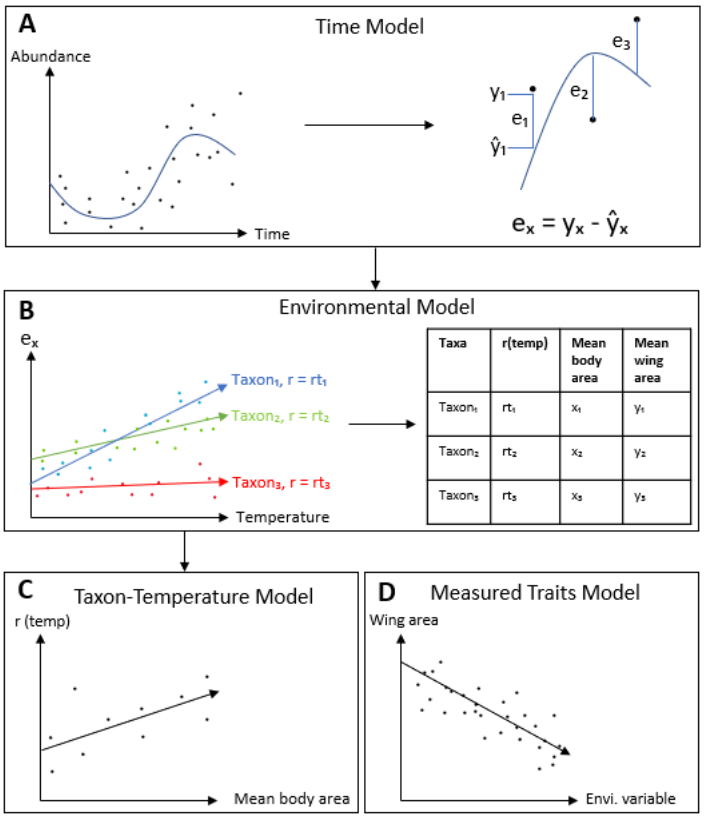

Figure 12. The flow of information between models used in the High-Resolution Sampling study. ***A.*** *First, I built a* ***Time Model*** *describing insect abundance as a function of time.* ***B.*** *In a second step, I built an* ***Environmental Model*** *describing the residuals from the time model as a function of environmental variables * Taxon interactions.* ***C.*** *As a third step, I built a* ***Taxon-Temperature Model*** *describing the slopes from each Temperature * Taxon interaction as a function of each taxa mean wing and body area.* ***D.*** *In addition, I built a second trait model (referred to as the* ***Measured Traits Model****) describing how measured trait values vary as a function of the environmental variables regardless of taxon-identity. Note that all data points and trendlines in the figures are arbitrary and used for illustration alone.*

Second, to establish whether the activity of insects could be explained by physical traits, regardless of taxon identity, I built two linear mixed effect model, hereafter called Measured Traits Models, using the wing area or body area from the 1554 measured individuals as a function of the environmental variables (Fig 12D; Table 2.7, 2.8). To account for the fact that individuals caught within the same sampling period do not constitute fully independent data points, I used the specific time of sampling as a random factor.

Table 2. An overview of models fitted to data from different experiments, with their distributions and links. For each model the question, type of model, R-function and R package used is given. Response variables in italic. '' denotes interaction terms. "Days since treatment", "Wind speed", "Temperature", "Cloud cover", "Relative Humidity", "Wing area", and "Body area" were defined as continuous explanatory variables, "Amount" as an integer, and all others as factors.*

Experiment	Question	Model type: function (R package)	Model	Distribution	Link
1. Survival	Does survival over time differ between UV-fluorescent color treatments and does this effect depend on species?	Generalized linear mixed model: glmmTMB (glmmTMB)	*Individuals alive/Individuals Dead* ~ Color treatment*Species*Days since treatment + (1 |Treatment:Subtreatment) + (1|Row ID)	Binomial	Logit
2. Flight	Does flight ability differ between UV-fluorescent color treatments and does this effect depend on species?	Generalized linear mixed model: glmmTMB (glmmTMB)	*Individuals recaught/Individuals not recaught* ~ Color treatment*Species	Quasi-binomial	Logit
3. High-resolution sampling: Time model	Does abundance of insects caught in malaise traps vary with time of day, and is this effect different depending on taxon identity?	Generalized linear mixed model: glm.nb (MASS)	*Amount* ~ Taxon + sin(2*π*Time of day/24) +cos(2*π*Time of day/24) + (1|Date)	Negative binomial	Log
4. High-resolution sampling: Environmental model 1	Can environmental variables further explain variation in abundance unexplained by the time model?	Linear mixed model: lmer (lme4)	*Log(Time model residuals)* ~ Temperature * Taxon + Cloud cover * Taxon + + Wind Speed * Taxon + Relative Humidity * Taxon + (1|Date)	Gaussian	Identity
5. High-resolution sampling: Environmental model 2 (chosen for analysis)	Does the Environmental model improve (= higher AIC score) if we remove non-significant terms?	Linear mixed model: lmer (lme4)	*Log (Time model residuals)* ~ Temperature * Taxon + Cloud cover + Relative humidity + Wind Speed + (1|Date)	Gaussian	Identity
6. High-resolution sampling: Temperature slopes – Mean traits	Does the taxon-specific effect of temperature on abundance vary with the trait values of the taxa?	Linear model: lm (Base R)	*Temperature * Taxon slopes* ~ Wing area + Body area	Gaussian	Identity
7. Wing area - Environment	Do insects favor certain environmental conditions depending on wing area?	Linear mixed model: lmer (lme4)	*log(Wing area)* ~ Temperature + Cloud cover + RH + Wind speed + (1|TimeDay)	Gaussian	Identity
8. Body area - Environment	Do insects favor certain environmental conditions depending on body area?	Linear mixed model: lmer (lme4)	*Log(Body area)* ~ Temperature + Cloud cover + RH + Wind speed + (1|TimeDay)	Gaussian	Identity

Results

Survival experiment

The UV-color treatments affected species survival differently over time (Fig 13). This was revealed by a significant interaction effect between species, color treatment, and day since color application on the survival of the mass-reared species *(D. littoralis, D. simulans, E. balteatus*, *O. majusculus, M. pygmaeus,* and *D. isaea*) (Table 3). An identical model focusing on only the first five days post-treatment gave similar results as the model examining the entire periods (Table 3).

Table 3. Analysis of Deviance tables from type III Wald chi-square tests on models looking at population survival against color treatment, days since treatment interaction and species. Two models are shown: one of data from the full experiment and the other only the first five days post-treatment. Since my prime interest was in the differences in survival over time between treatments within the different species, I provide P-values for three-way interactions between treatment, day since treatment application, and species.

Model	**χ^2**	**Residual deviance**	**df**	**p-value**
5 days post-treatment	46.1684	1755.4	383	5.0e-05
17 days post-treatment	157.146	3266.0	811	2.2e-16

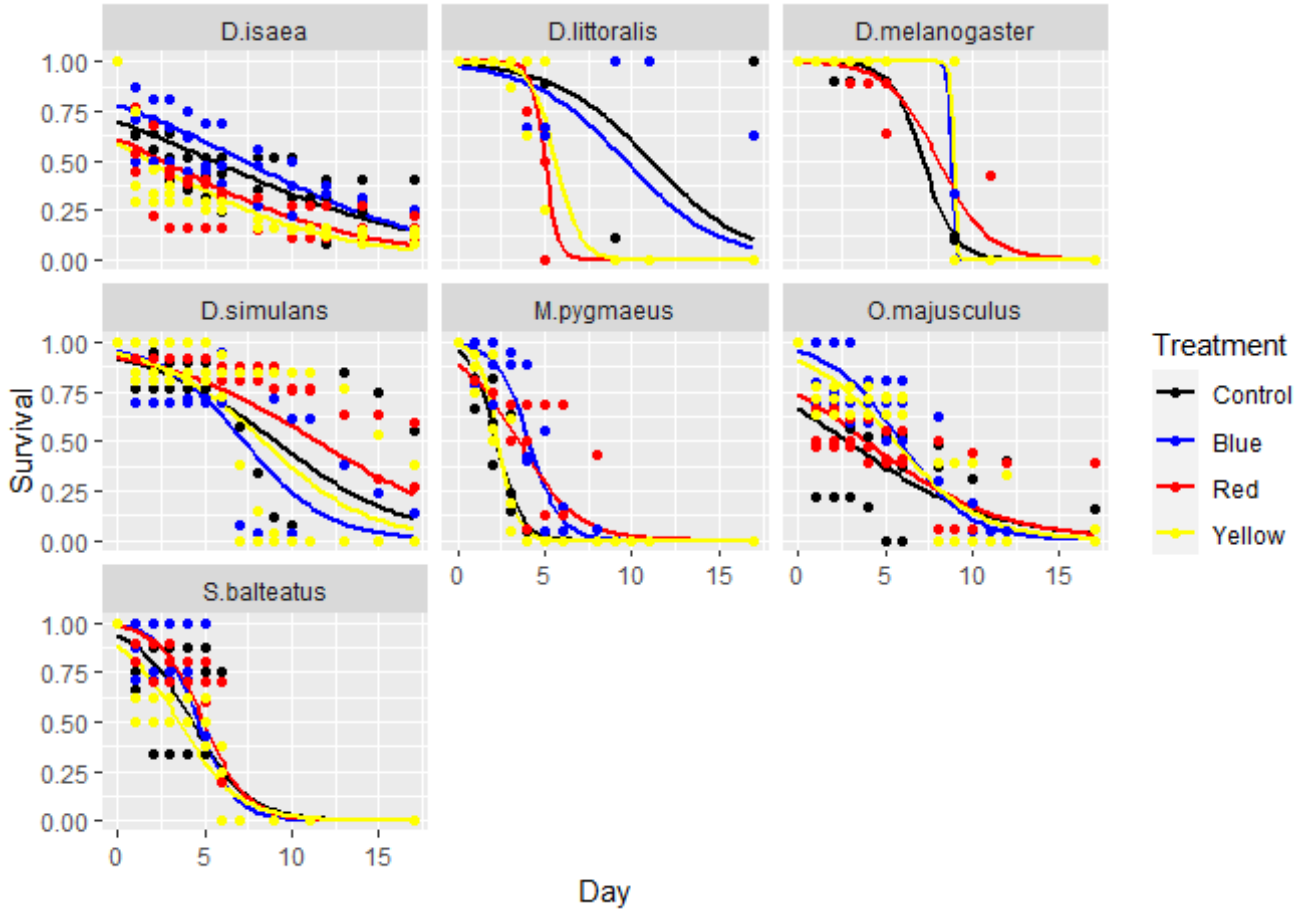

Figure 13 Percentage survival of populations over time, per species, across populations marked with three different UV-colors, along with unmarked control groups. Survival is given as a percentage of initial population size, found alive at the current time-step. Seven mass-reared insect species were used - D. melanogaster, D. littoralis, D. simulans, E. balteatus, O. majusculus, M. pygmaeus, and D. isaea. Note that D.melanogaster was removed from analysis due to complete separation of data (i.e., no overlap in dates between observations of survival and death). Dots are raw data points, lines show fitted glm:s.

Flight experiment

I used mass-reared species *S. ruepellii, E. balteatus, D. melanogaster, D. littoralis, D. simulans, M. pygmaeus,* and *D. isaea* to examine the effect of color treatment on flight ability. When examining the effect of treatments (Fig 14), we see that the mean effect of each treatment falls within the confidence interval of the control groups' effect. This pattern was similar across species, as examined by the interaction Species * Treatment (Table 4). There was, however, a significant effect of species (Table 4, Fig 15), implying that mean survival differed among taxa across the experiment, however not among treatments.

Table 4. Analysis of Deviance table of a GLM model looking at individuals recaptured, per species, on yellow st icky traps after color treatment applications, as a function of color treatment and species. Residual deviance = 77.984 on 28 degrees of freedom. Significant p-values in bold.

Model term	χ^2	df	p-value
Treatment	5.3546	3	0.1476
Species	29.6819	6	**<0.001**
Species*Treatment	15.5226	18	0.6258

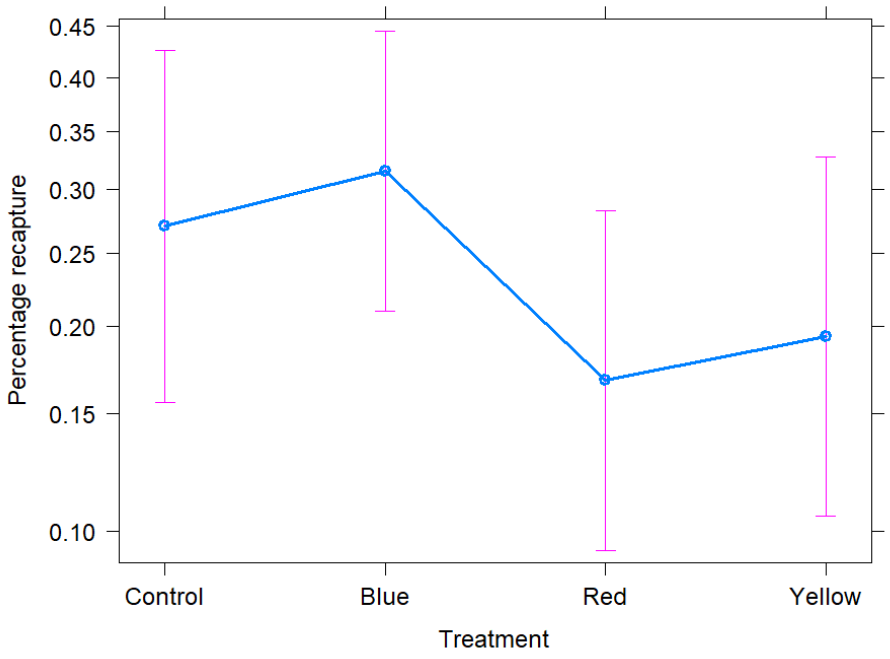

Figure 14. Effect plot of the different treatments against percentage of released, marked insects recaptured on yellow sticky traps. Blue horizontal line intercept mean values, pink vertical lines show confidence interval for each treatment group. The control group was not exposed to any color marking.

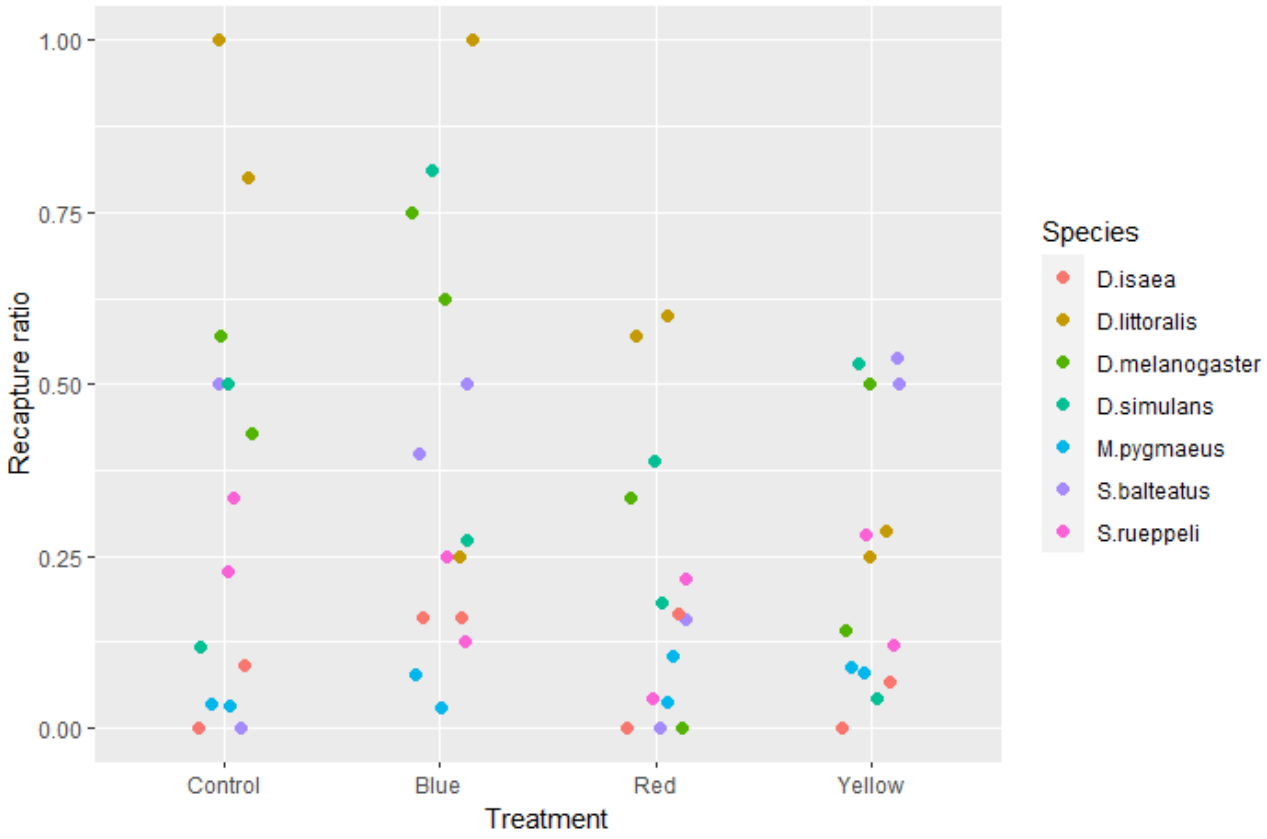

Figure 15. Flight ability of seven mass-reared insect species (S. ruepellii, E. balteatus, D. melanogaster, D. littoralis, D. simulans, M. pygmaeus, and *D. isaea) after being marked with either one of three different UV-colors, together with a control group. All species were released together within 56x70x36 cm mesh mesocosms. Flight ability was measured as percentage of individuals recaught within 48 hours on yellow sticky-traps in the mesocosms. The experiment was repeated twice, depicted as two separate points for each species and treatment.*

Mark-recapture experiment

In total, 6585 marked insects were re-released into the experimental area. 3930 of these were mass-reared in lab settings, the remaining 2655 were wild caught (Table 5). Of these, 119 individuals, amounting to 1.8%, were recaptured. However, no mass-reared insects were recaptured. As a result, the 119 recaptured individuals all came from the 2655 individuals caught and marked in-situ. This brings the recapture rate of insects sampled from natural populations to roughly 4.5%.

Table 5. The number of released individuals and recapture rate for the total number of released individuals, mass-reared individuals, and wild-caught individuals.

Type of insects	Number of individuals released	Recapture rate
Total released	6585	1.8%
Mass-reared	3930	0%
Wild-caught	2655	4.48%

High-Resolution Sampling

The daily rhythms of the different taxa were in many cases well described by the sine and cosine curves, as seen by the plots (Fig 16). Furthermore, the different taxa exhibited different circadian rhythms (Table 6). Most taxa tended to peak in abundance between 12:00-22:00. However, *Microlepidoptera* and *Throscidae* showed a clear nocturnal pattern, peaking between 22:00 and 02:00 (Fig 16). In general, the taxa deviated from predicted patterns by one half to three times the predicted abundance at each timestep.

Table 6. Analysis of Deviance table from a GLMM evaluating the interaction effect of daily rhythm, expressed as a cosine and sine curve respectively, and taxon on abundance of insects found in malaise traps. Only the 17 most common taxa were used in the model.

Model term	Residual deviance	df	p-value
*Taxon * Sin(2*π*Time/24)*	*993.05*	*16*	*<0.001*
*Taxon * Cos(2*π*Time/24)*	*855.29*	*16*	*<0.001*

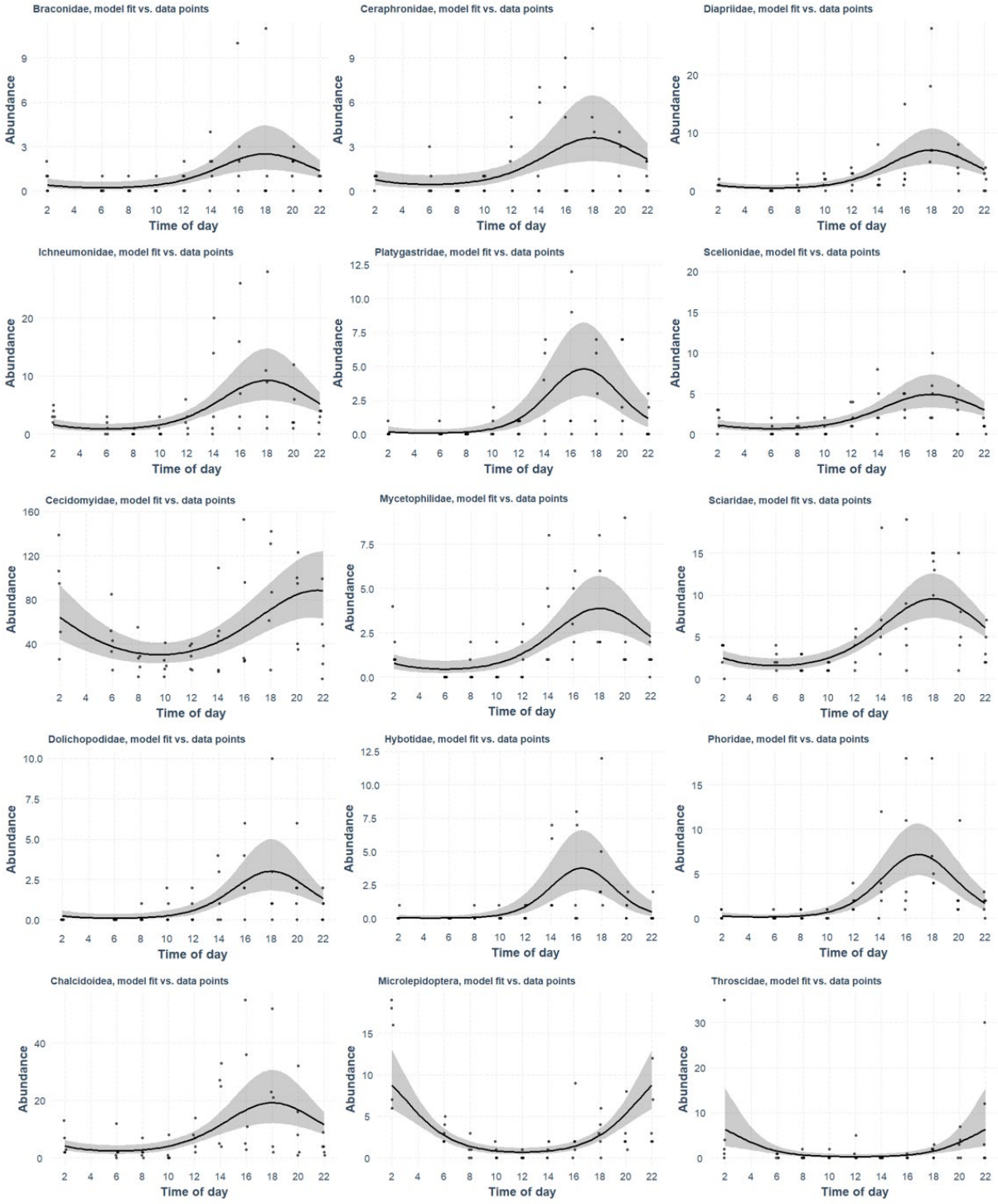

Figure 16. Abundance of individuals against time of day for each of the 17 most common taxa found in the Malaise traps during the High-Resolution sampling. Dots represent raw data from each of five days of sampling per hour of measurement. Lines depict model fit from the Time Model employing a sine and cosine function of time.

Using the residuals from the abovementioned Time Model as a measure of unexplained variance, I could see that increased wind speed and cloud cover, respectively, resulted in lower abundance of insects across all taxa (Table 7; Fig 17). There was also a tendency towards a positive imprint of relative humidity, although this effect was not statistically significant. For temperature, all taxa increased in abundance with increased temperatures, however the strength of this increase in abundance varied between the taxa (Fig 18). The relationships between temperature and abundances for each taxon were not further explained by mean body area. However, the relationship between temperature and abundance did shrink with an increase in wing area, as evaluated by linear models (Table 8; Fig 19).

Table 7. Analysis of Deviance table from a GLMM evaluating the effect of environmental variables on the residuals from the time model, i.e., testing their independent effect on abundance on insects caught in the malaise traps. '' denotes interaction terms. Estimates of correlation coefficients, along with standard errors, are given for the continuous variables. Significant p-values in bold.*

Model term	Estimate	Std. error	χ^2	df	p-value
Taxon	-	-	13.90	16	0.606
Temperature	0.041	0.072	0.318	1	0.573
Cloud cover	-0.064	0.019	11.33	1	**<0.001**
Wind speed	-0.128	0.023	32.15	1	**<0.001**
Relative Humidity	-0.029	0.031	0.888	1	0.346
Temperature * Taxon	-	-	32.31	16	**<0.01**

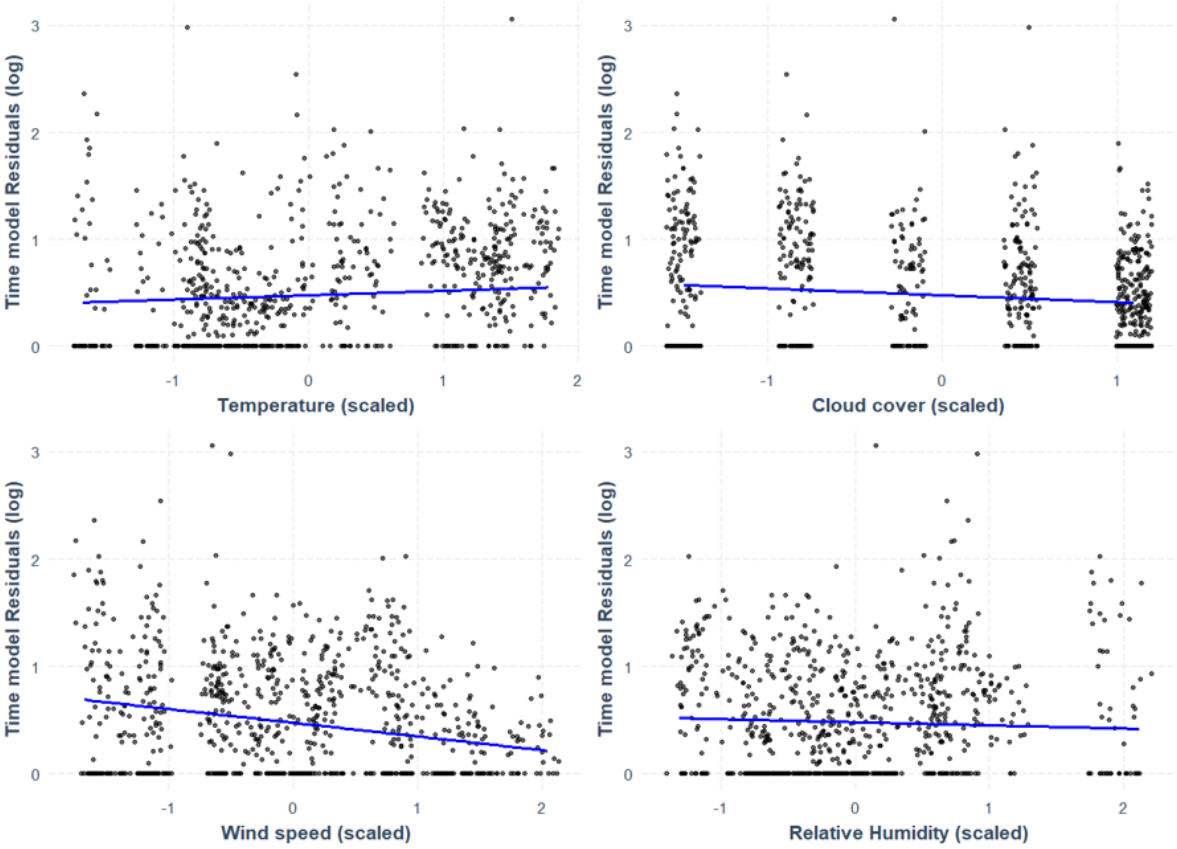

Figure 17. The effect of the different environmental variables on the residuals from the time model, i.e., their independent effects on abundance of insects caught in malaise traps. All explanatory variables are scaled, Time model residuals are log-transformed. Dots depict raw data; lines show model fit.

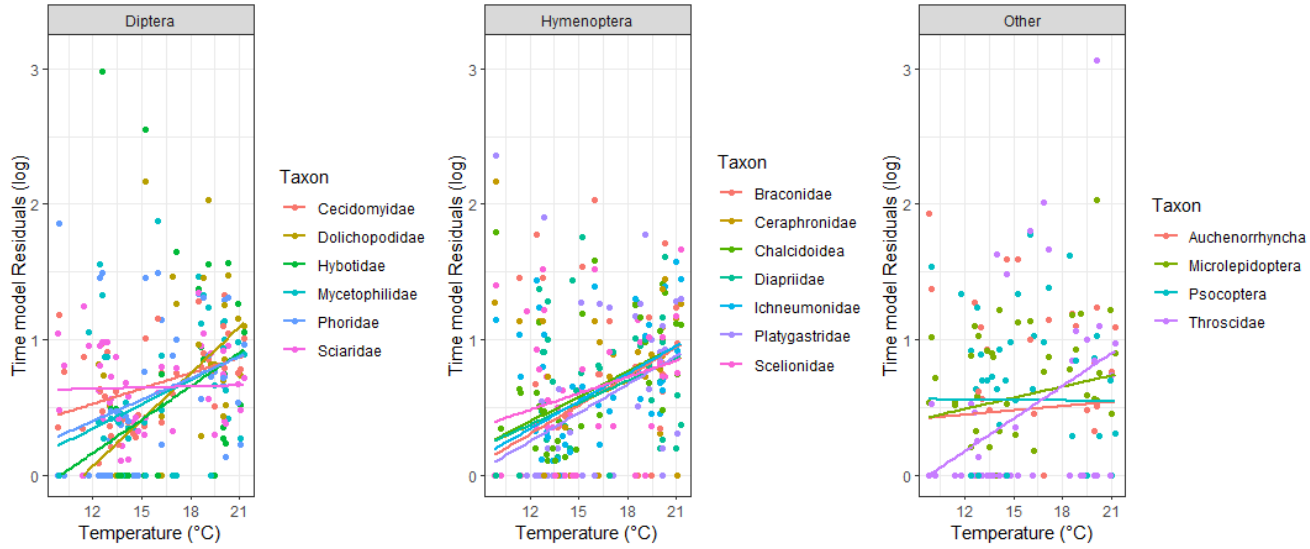

Figure 18. The effect of temperature on log-transformed Time Model Residuals, per taxon. The graph is divided by taxonomic Order, showing, from left to right, taxa of Diptera, Hymenoptera, and a mix of taxa from other Orders. Dots depict raw data; lines show linear relationships. Note that these plots do not take the effect of other environmental variables into account. The Time Model residuals are on a log(x + 2) scale to avoid negative values. Thus, a value of 0 corresponds to exp^(0) – 2 = (-1) (Activity lower than expected), and 1 corresponds to perfect fit (temperature had no effect on abundances).

*Table 8. Analysis of Variance table for linear models with the environmental variable * Taxon slopes from the environmental model as a function of mean taxon wing area, or mean taxon body area. See Fig. 12 for further explanation of how I obtained slope values. R^2 depicts explained variance, and 'estimate' the correlation coefficients along with their standard errors. Significant p-values in bold.*

Response variable	Explanatory variable	R^2	Estimate	Std. Error	p-value
Temperature*Taxon slopes	Mean taxon wing area	0.430	-0.175	0.055	**<0.01**
Temperature*Taxon slopes	Mean taxon body area	0.430	0.031	0.040	0.448

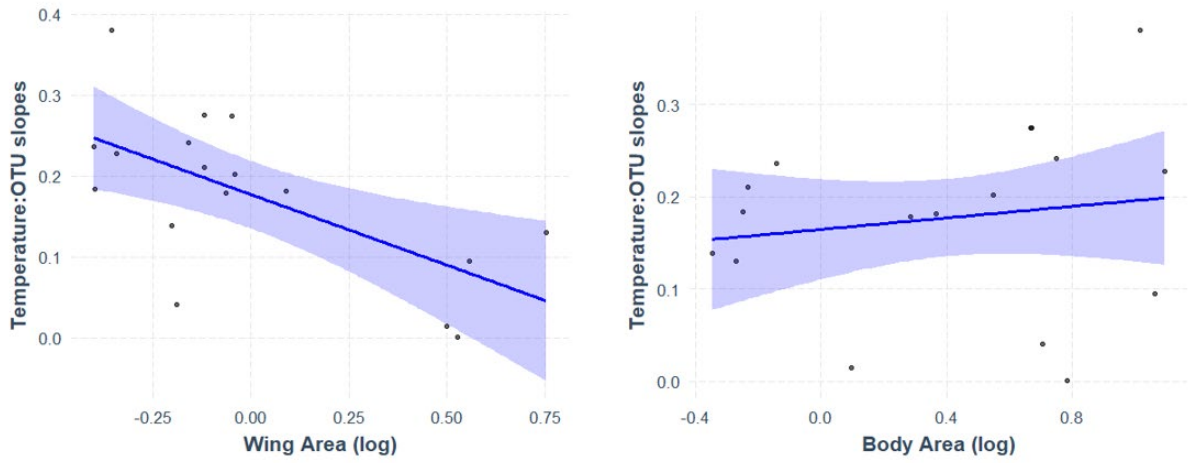

*Figure 19. The effect of traits (mean trait value per taxon) on the slopes between Temperature * Taxon interactions from the Environmental Model. Points depict raw data; lines show model fit with confidence intervals.*

All environmental variables except relative humidity (Temperature, wind speed, cloud cover) showed a negative correlation with wing area (Fig 20). That is, the lower the value of the variable, the bigger the wing area of the insects caught in the Malaise traps. However, only temperature and wind speed showed such a relationship strong enough to be statistically significant (Table 9). Body area was uncorrelated with all environmental variables, as shown by the weak trends and unsignificant relationships (Table 9; Fig 21).

Table 9. Estimates of correlation coefficients and their standard errors for GLMM:s evaluating models with trait values as a function of environmental variables. I obtained p-values from analysis of deviance tables. Significant terms in bold.

Response variable	Explanatory variable	Estimate	Std. error	p-values
Wing area	Temperature	-0.073	0.039	**<0.05**
Wing area	Cloud cover	-0.002	0.034	0.996
Wing area	Wind speed	-0.071	0.033	**<0.05**
Wing area	Relative humidity	0.061	0.054	0.257
Body area	Temperature	-0.131	0.091	0.150
Body area	Cloud cover	0.077	0.099	0.435
Body area	Wind speed	0.065	0.098	0.505
Body area	Relative humidity	-0.159	0.156	0.309

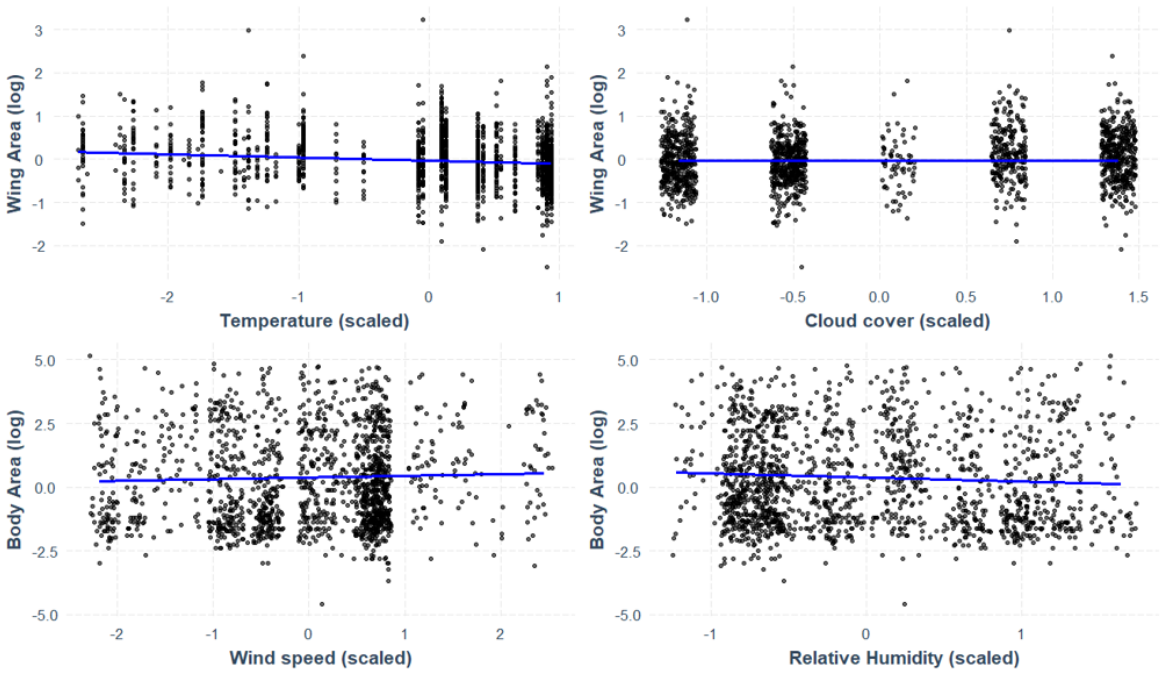

Figure 20. Each environmental variable (scaled) against wing area (here log-transformed) from the 1554 measured individuals. Black dots show raw data, blue lines depict model fit.

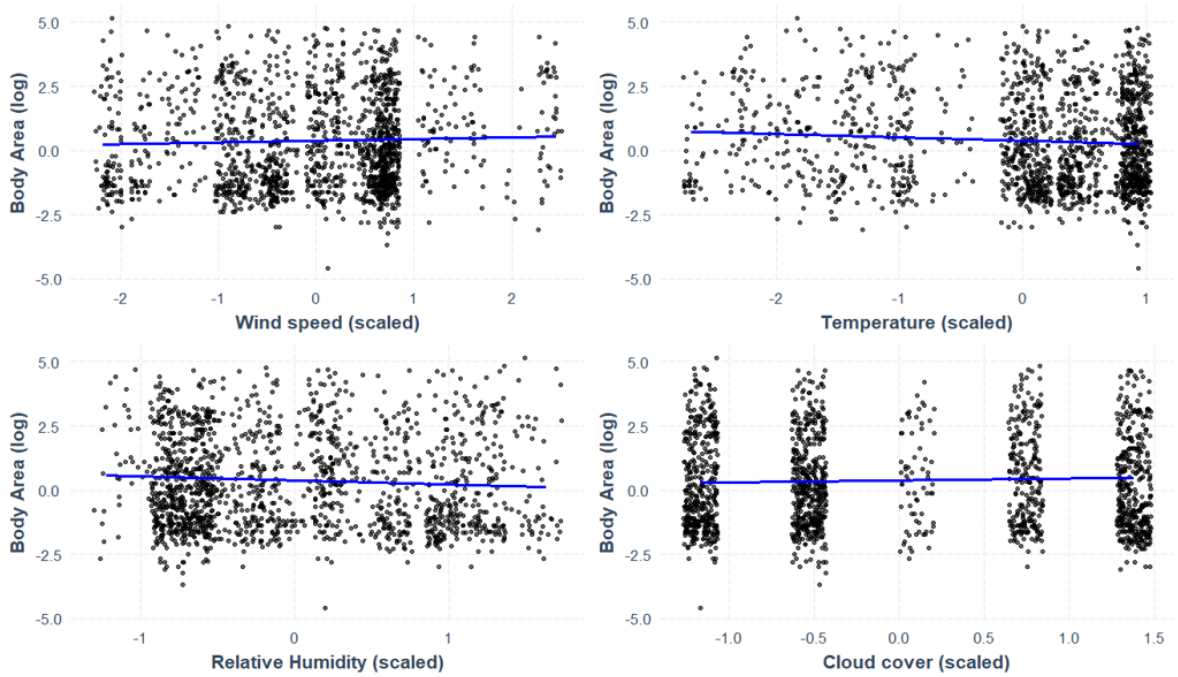

Figure 21. Each environmental variable (scaled) against body area (here log-transformed) from the 1554 measured individuals. Black dots show raw data; blue lines depict model fit.

Discussion

In this thesis, I aimed to dissect how weather affects the activity patterns of insects in relation to Malaise traps. I also aimed to develop a method for mark-recapture in Malaise traps for future studies on subsequent movement patterns of these insects. With knowledge of how activity through weather conditions and movement patterns affect a taxon's detectability, one can account for this detectability when relating catches to real densities, or address biases caused by differences in detectability between taxa.

To perform a mark-recapture study of insects in Malaise traps, I had to develop a new method for mass-marking live insects and validate that this method had no negative impact on survival and flight ability. To relate insect activity (and thereby abundance in flight) to environmental conditions, I sampled Malaise traps with short intervals and compared fluctuations in abundance from expected patterns of daily rhythm with fluctuations in the environment. Below, I will examine my findings for each step in this chain.

A new method for mass-marking of insects

To quantify mark-release-recapture experiments, I developed a new method for bulk marking of insects. This method was built on previous approaches by Stern & Mueller (1968), Dickens & Brant (2014), and Culbert *et al.* (2020). Nonetheless, to validate that the fluorescent dusts used by me did not affect survival or flight ability, I had to conduct several experiments.

In terms of the fluorescence dusts used, I did find some slighter effects of the specific product used in terms of insect survival. The specific effect of color treatment on survival of different mass-reared insects over time varied with both time since treatment and species. Due to this three-way interaction, the relative difference between the different color treatments and the control groups will depend on what time perspective we adopt. In *D. littoralis* and *D. isaea,* red and yellow treatments indicated a small decrease in survival compared to control. In most other species however, the survival of red and yellow treatments followed the survival of control groups. Only in *D. simulans* did the blue treatment correspond to highest death rates. In conclusion, there is no sign that any one color would have a significant negative impact on survival, compared with the control, across all species. Using these UV-colors to mark insects for mark-recapture experiments should thus have a marginal impact on the insects' survival.

In line with the limited effects on survival, the fluorescent dusts' effect on flight ability did not significantly differ between colors, or between insects treated with dusts and untreated control groups. Furthermore, since there was no significant interaction effect between color, treatment and species, this lack of effect of insect dusting on flight ability seems to be constituent across species. It is thus reasonable to say that, to the best of our knowledge, applying these fluorescent-dust treatments to insects does not significantly hamper their flight abilities. This conclusion, together with the conclusion from the survival experiment, leads me to conclude that applying these color treatments on insects for mark-recapture will not affect the insects' ability to move around the landscape once released.

With the marking methods developed, I was able to implement a major mark-release-recapture study on insects of small to very small sizes. Mean recapture rates from other

studies using attractive trapping have fluctuated between 3-10% (Saul 1987, Arellano *et al.* 2008, Robinet *et al.* 2019). Active search provides higher numbers, however it requires more effort and comes with a less standardized methodology (e.g., Öckinger & Smith 2008, Tikkamäki & Komonen 2011). In relation to this, a 4.5% recapture rate using my passive marking device, requiring no human interaction with the insects, and recapturing in entirely passive traps, must be deemed as good and the method a subsequent success.

To optimize this marking design, better control of moisture and temperatures inside the marking devices (Fig 4) would be favorable. While there is no data on these variables, nor the total amount of individuals that died during marking, I observed during the experiment that high temperatures especially during afternoons caused a death rate of roughly 30-70% inside the devices. On days with heavy overcast or rain, the death rate came close to zero, although the capture rates were many times lower than on warm days. Thus, a method to increase survival on warm days would increase the number of marked individuals ready for release substantially. Based on personal observation I would also recommend conducting sampling at forest edges, rather than inside forests. The captures rates of insects for marking at traps inside the forest were always substantially lower than for the traps at forest edges. It should however be noted that while edges are likely to contain a more diverse and abundant community, the composition might differ somewhat from the composition inside the forest (Nguyen & Nansen 2018). Mark-recapture of edge-prone insects in a homogenous landscape might thus provide slight behavioral biases compared to using insects naturally found within that homogenous patch.

The lack of recaptured individuals from lab-reared populations offers a clear comparison to the use of natural populations, which showed a recapture rate of 4.5%. Earlier studies looking at the potential of marking mass-reared species have conducted their work in lab-setting, asking for studies attempting to replicate their work in natural settings (Dickens & Brant 2014; Culbert et al 2020). While my study did not investigate the possibility of using mass-reared species for mark-recapture *per se,* the total lack of recaptures, especially in comparison to the recapture rate of insects from natural populations, points towards mass-reared insects lacking the ability to behave in a natural manner once released. In effect, my results indicate that studies using mass-reared species for mark-recapture experiments risk biased results at best, with a high chance of ending up without any results whatsoever.

To summarize, my mark-recapture design proved an effective method for obtaining data on movement patterns of very small insects, or for assessing total populations sizes, in relation to Malaise traps. Such movement data could then be related to average diffusion rates of insects dependent for example on taxonomic identity. Recapture distance from release point, coupled with time between release and recapture, provide information on diffusion rate per time unit. This knowledge can then be used to make inferences regarding insects' movement activity in relation to taxonomic identity.

Factors affecting the short-term flight-activity of insects

By conducting repeated sampling with short intervals around the clock, I was able to tease apart the impact of circadian patterns in activity from the effects of contemporary environmental conditions on the flight activity of insects. As expected, all taxa used in the analysis showed a strong daily rhythm. Of the different taxa, Microlepidoptera and

Throscidae were clearly nocturnal, *Cecidomyiidae* was mostly active from late afternoon throughout the night, and all other taxa were either afternoon or dusk active. Furthermore, all day-active groups peaked between 14:00-20:00, in accordance with results from a similar study of insect activity by Genoud *et al.* (2021). This correlates with the peak in daily temperatures during the study period, making it near impossible to distinguish between the effect of the two. Do insect activity peak in the afternoon because of their circadian rhythm, or because daily temperatures peak in the afternoons, or due to a mix of both? In total, abundances varied between being three times higher to one half lower in relation to expected values from the daily rhythms.

When employing a Malaise trap for sampling, the goal is usually to sample taxa that commonly occur in Malaise traps, such as those mentioned earlier. The taxa I found to be most numerous in my traps seem to be the most commonly found taxa in Malaise traps in Sweden (Karlsson *et al.* 2020). The only deviating taxon was *Chironomidae,* which tend to be very common yet were surprisingly scarce in my samples. This clear link between the composition in my samples and samples from other studies further shows that my results can be extrapolated to Malaise traps in Sweden in general, and most likely to other countries in similar climatic zones (Fraser *et al.* 2008, Matthews & Matthews 2017), or even in completely different climatic regions (Brown 2005). Different habitats may however contain insects adapted to different weather conditions, and this could in turn make the results seen here non-applicable to other habitats. However, most insects are likely to react in the same manner to increased temperatures and wind speeds, independent of local adaptations. In effect, species may be adapted to higher activity during cool temperatures and high wind in certain locations, yet they are still likely to be more active as temperatures rise and wind speeds subside (Digby 1958, Heath *et al.* 1971, McGeachie 1989, Mellanby 1997, Danks 2004).

Furthermore, I would argue that many other taxa found in small numbers should be discarded in regard to detectability analyses. Some of these taxa are more efficiently sampled using other trapping methods, such as active search with nets or passive pan traps for pollinators (Popic *et al.* 2013). These taxa would thus not be subject to extensive sampling through Malaise traps and are therefore not relevant for detectability analyses. Furthermore, some scarce insects may be attributed to transient species (insects that move across large areas and encounter the trap by chance) rather than part of the local fauna. Due to this, their presence should, in my opinion, be of little relevance when assessing local community compositions and can thus be ignored (Steinke *et al.* 2021). Altogether, I believe there are robust arguments for ignoring scarce insects in detectability analysis, making my group of the 17 most common taxa chosen for analysis a well-supported choice of delimitation.

Temperature

That temperature had a positive effect on most taxa caught in Malaise traps comes as no surprise, as many previous studies have shown that temperature is the main explanatory variable of insect abundance (Peng *et al.* 1992, Briers *et al.* 2003, Genoud *et al.* 2021). Insect activity usually varies between an upper and lower threshold (Williams & Osman 1960, Taylor 1963, Heath *et al.* 1971, Johansson *et al.* 2020), remaining fairly constant in activity within that threshold, with subsequent lower activity whenever above or below it. This would result in abundance behaving in a manner of logistic growth against temperature. I saw no such trends in my analysis. However, during my sampling period, the temperatures varied

only between 10-22°C. It is thus likely that during the relatively short duration of my study, temperatures never reached values high enough to exceed the upper threshold of activity. If we instead turn to lower thresholds, my results showed an indication of increase in overall insect activity when temperatures reach 15 degrees. From a trapping perspective, this would mean that when trapping on days where temperatures does not reach above this threshold, catches could be skewed in relation to true densities due to low activity. However, there is no *proof* of such a threshold here, merely an indication. While there was a higher ratio of low abundances at low temperatures, there was also an equal spread of high abundances across all temperatures, which argues against such a threshold.

Focusing on deviations from expected circadian patterns, the Environmental Model revealed a strong temperature * taxon interaction, which translates to a clear difference in the response of different taxa to prevailing temperature conditions. Nonetheless, a visual assessment of these relationships further showed that almost all taxa exhibit a strong positive correlation with temperature. Especially so for the most common taxa here – families of *Parasitica* (including superfamily *Chalcidoidea),* Microlepidopterans, *Phoridae,* and mosquitoes of superfamily *Sciaroidea* (Including *Cecidomyiidae, Sciaridae,* and *Mycetophilidae).* All these taxa showed a clear increase in abundance with temperature except for *Sciaridae,* which appeared to be unaffected by temperature. So, while temperatures during sampling may affect the number of insects found in the trap, the composition of the most common taxa will remain almost unchanged.

The taxon-specific reaction to temperature was also strongly correlated with mean wing area. The larger the wing area in relation to the expected wing: body ratio, the lower the effect of temperature on the activity of that taxon. This implies that certain traits play an important role in determining activity, and this effect could be intertwined with effects of taxonomic identity. In effect, do taxa with greater-than-expected wing area react less to changes in temperature because of that trait, or because they, through evolutionary kinship, share an ability to be active across temperature fluctuations independent of wing size? Or because of a mix of the two explanations? Furthermore, temperature affected trait-value specific activity dependent on wing area, however there was no such effect seen for body area. Insects with large wings in relation to the expected wing: body ratio were common at low temperatures, and at high temperatures insects with smaller wings than anticipated in relation to expected wing: body ratio became more common. However, as discussed above, while mean wing area thus seems to influence the insect activity in relation to temperature, the most common taxa found here all showed similar increases in abundance in relation to temperature. This trait-specific effect might then be of more importance if one would be interested in sampling specific species or genera, rather than a factor to consider when sampling full communities.

Wind speed

Wind speed had a strong overall effect on insect activity. Furthermore, this effect was similar across taxa. I find this surprising, as wind speeds were generally low (0.5-4.7 m/s) and measured at a meteorological station approximately 10 kilometers away. It is likely that this station was placed in a more exposed environment than the Malaise traps, which would result in lower-than-expected wind speeds around the traps. Even so, wind speed had a significant negative effect on abundance. Assuming lower real wind speeds than expected from data, it may thus be that the true effect of wind speed is even greater than measured here. However,

as mentioned there was no difference in abundance shifts between taxa, implying that wind does not subset catches by taxon identity.

A study of *Plecoptera* activity in relation to environment, assessed by Malaise trap catches, found no effect of wind below 2 m/s and the overall effect of wind to be much smaller at wind-protected sites (Briers *et al.* 2003). Again, this contrasts with the current results. *Plecoptera* are usually big insects, and a larger body mass could correspond to less effect of wind at low wind speeds. However, I found no effect of body area on abundance in relation to wind speed. This, together with the non-significant interaction between wind speed and taxon belonging, also means that very small insects show no difference in reaction to wind compared to large insects. Despite there being no trend between body area and wind speed, there was such a trend between wing area and wind speed. In effect, a shift in wind speed from below average (<2.4 m/s) to above average (>2.4 m/s) resulted in a shift from insects with higher-than-normal wing: body ratio being more common, to insects with lower-than-normal wing-to-body ratios being more common (Fig 20). This, however, does not correspond to insects with lower-than-normal wing: body ratios appearing in higher numbers at higher wind speeds. Rather, it seems insects with higher-than-expected wing: body ratios become less abundant at higher wind speeds.

Cloud cover

Insect activity decreased with cloud cover, which was in line with expectations. However, the effect did not differ between taxa or trait values. In effect, cloud cover may change the total amount of insects caught, however it will not influence the relative ratios of the different taxa (accounting for the most common taxa in Malaise samples). This is interesting, as earlier studies have claimed such effects should be present. Willmer & Unwin (1981) and Herrera (1990), all argued that higher solar radiation should favor the activity of small insects, while big insects should refrain from direct sunlight when temperatures are high to avoid overheating. However, very large insects such as bumblebees and certain beetles have a tendency to drop when encountering a Malaise trap (Matthews & Matthews 2017), effectively avoiding being sampled. Furthermore, as previously mentioned, temperatures during the study period never reach levels high enough to indicate possible overheating and thus a tendency for large insects to lower activity.

While there is likely a correlation between cloud cover and temperature, where cloud cover decreases temperatures by day and increase them by night, cloud cover may also provide a unique effect by inhibiting solar radiation. If we accept the reasoning by (Williams & Osman 1960, Taylor 1963), that there is an upper threshold for activity above which the risk for overheating becomes too large for an insect to dare fly, then a decrease in solar radiation, equivalent to insects finding shaded shelter, should increase activity despite of high temperatures. The effect of solar radiation, through cloud cover, should thus depend on the temperature. My results point toward less activity with higher cloud cover. In this case, this can be attributed to lower solar radiation at low temperatures. Since temperatures never reached a point to suggest any kind of upper threshold for activity, cloud cover should be expected to only exhibit a negative effect on insect abundance, as seen here.

Relative Humidity

Relative humidity had no significant effect on insect activity, despite its use in navigation for many species (Enjin 2017). Previous work has identified humidity as an important

environmental variable in explaining insect activity for Dipterans (Peng *et al.* 1992). However, for other insect groups humidity seem to have a neglectable effect on activity (Williams 1940, Briers *et al.* 2003). It is noteworthy that I used the residuals from the linear relationship between relative humidity and temperature as an independent measure of relative humidity. I did this because warmer air can carry more water vapor (Lawrence 2005), whereby one and the same absolute humidity will result in different relative humidities with a simple change in temperature. The effect of humidity on insect activity and behavior is related to their water content (Bursell 1974). By controlling the amount of water in the air, and thus the degree of desiccation in the insects, temperature will contribute to the magnitude of this effect. The positive effect of relative humidity found by Peng *et al.* 1992 might then have been attributable to its correlation with temperature. The effect of relative humidity on activity may, however, also differ depending on in which type of habitat one samples, as insects' adaption to humidity varies with habitat preferences (Heath *et al.* 1971, Danks 2004, Enjin *et al.* 2016).

Summary of environmental impacts on insect activity

The effects of environmental conditions on the abundance and composition of insects in Malaise traps (i.e., their effect on detectability) are gathered in Table 10. All in all, these dependences on physical conditions regarding what is observed in the trap occurred at a time frame much faster than phenological turnover, or even marked demographic turnover (i.e., new individuals being born/hatched, or dying) in the focal community. In other words, environmental conditions will affect the relation between the community to be described, and the Malaise-based sample thereof. In terms of abundances, these effects are large, since the number of insects found can vary from less than half to three times as many as expected from daily rhythms alone. These fluctuations suggest that our estimate of local community size may be significantly affected by the conditions prevailing during the trapping period. However, these weather conditions will not alter the taxonomic composition of the samples, at least not for the most numerous taxa. Body size did not influence how insect activity responded to weather. While reaction to wind speed and temperature was connected to wing size of active individuals, there was no difference in effect of wind speed between taxa, and a neglectable relationship for temperature when focusing on the most numerous taxa. The effects of weather on trait-specific activity may thus be more prominent for less common insect groups, which I have already argued are of less importance in quantitative Malaise studies. It should also be noted that Malaise traps typically are sampled over a timespan of week or weeks. Any effects of weather on abundances at one time step may then be cancelled out by an increase in abundances at another day. While the weather during the insects' active periods each day will contribute to the overall abundances found after a week, the effect of weather on composition may then not be as prominent as found here. I thus believe that my results can be simplified to the conclusion that weather conditions can have a large effect of overall insect numbers in the traps, however changes in composition will be neglectable.

While this study used only one site, a young forest dominated by Scots pine, the composition of taxa found during the sampling period are in line with what other studies have found in Sweden (Karlsson *et al.* 2020), and in other parts of the world (Brown 2005). The effects of weather on activity are also in line with results from many earlier studies (Williams 1940, Briers *et al.* 2003, Genoud *et al.* 2021). It is known that even within habitats, a higher number of traps spaced over a larger area would provide more species, which may affect the results

(Steinke *et al.* 2021). However, considering that my catches and results are in line with earlier work, I believe that the conclusions drawn here can be applied on a broader scale, albeit with certain caution.

Table 10. The studied environmental variables and their individual effects on overall abundance and composition of insects found in Malaise traps. + = Increase, - = Decrease, 0 = no effect.

Variable	Effect on abundance	Effect on composition
Temperature	+	May subsets catches based on the insects' wing area and their taxonomic belonging
Wind speed	-	Subsets catches based on the insects' wing area
Cloud cover	-	0
Relative humidity	0	0

To summarize, weather conditions during sampling will alter the abundances of insects caught in Malaise traps. Temperature and wind can also change the composition of samples. However, these changes in composition seem to be more prominent for rare insects and are likely to be small when considering a sampling period over several days. For the most numerous taxa found in the traps, there was no change in composition depending on weather. Other taxa that are common in Malaise trap samples, yet were scarce in this study, are both phylogenetically and anatomically close to sampled taxa. Thus, there is no reason to believe that they would behave much differently. An exception could be Heteropteran taxa, which exhibit deviating morphology and kinship from taxa examined here.

Conclusions

In this study, I aimed to develop an understanding of detectability of insects in relation to Malaise traps, and to pave way for further inquiries in this area. Initially, I aimed to use an approach of observational models and find how each taxon reacted in relation to different environmental variables. However, I found that all numerous taxa reacted in a very uniform matter to changes in weather conditions. Thus, the conclusions became very straight-forward and perhaps of great relief to those using Malaise traps extensively in their research – Malaise traps are robust traps that will sample all existing, common taxa in the area in the same ratios, independent of weather conditions (provided there are no extreme weather events). However, if pure abundances are of value, caution should be taken. The results presented here will provide an understanding of how weather during the sampling period might affect these numbers.

In further studies of insect detectability in Malaise traps, adding the factor of movement patterns and diffusion rates will be crucial to building the observational models suggested in the introduction. If the diffusion rates would prove as uniform as the activity patterns, relating total catches to actual insect densities will prove an easy matter. The novel mark-recapture method presented here has shown to be a viable option to achieve this aim, and possibly

many other aims. I encourage everyone interested in this topic to pursuit the study of insect detectability, so that we finally may know how the thousands of Malaise samples gathered every year actually relates to surrounding insect communities.

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Popular science summary

In this study, my goal was to investigate how the behavior of insects affect their chances of ending up in a Malaise trap. Malaise traps are tent-like insect traps that are very efficient at catching small insects and need no human interference during the sampling period. They are extensively used in monitoring schemes and studies of insect diversity. However, little is known about the catches from these traps in relation to real insect communities around the traps. My aim was to understand this relation between trap catches and true insect densities by investigating how the insects' movement patterns and activity affect their chances of ending up in the trap.

I call the probability that a species ends up in a trap *detectability.* That is, what are the odds that you will notice that species if you sample where it is present? *Detectability* depends on three factors*:*

1. The total amount of individuals of that species in the area. A trap simply cannot catch more individuals than there are in the area.
2. The species' activity pattern. If the insects are not active when you are sampling, they will not fly by the trap and so they will never end up in the trap, even if they are present in the area.
3. The species' movement patterns. If an active insect flies often within a small area and at a low height, it has a bigger chance of ending up in the trap than an insect that moves in large areas and high above ground.

In this study, my aim was to find out if and how detectability is affected by weather, which was seen as the main factor governing activity patterns. In other words, will you get the same kind of insects if you for example sample during a warm, dry day as on a cold, windy day? To pave way for future studies also examining the effect of the insects' movement patterns on detectability, I also aimed to develop a method to measure the insects' movement rates.

To map the movement rates and patterns of the insects, I wanted to develop a mark-recapture method for these small and fragile species. A mark-recapture study involves catching individuals alive and marking them in some manner that they can later be identified again. They are then released, and a new batch of individuals are captured. The number of recaptures of marked individuals can then be used to see how fast they move from the release point and in which directions. The number of marked individuals against the number of unmarked individuals also gives an approximate amount of total amount of individuals in the area. To be able to perform such a study with insects in Malaise traps, I had to develop a device that caught insects alive in the traps and marked them with a fine powder that lit up under UV-light. To do this, I first had to test if the UV-powder had any negative effects on the insects. I reared seven insect species in a lab and marked them with three different colors of UV-powder. I then looked at the survival of the insects and compared it to insects of the same species that had not been marked. I applied the same procedure to test the UV-powders effects on flight ability. I released marked and unmarked insects inside a cage with sticky traps and left them for 48 hours. The sticky traps could only be reached by flying. I then compared how many marked and unmarked insects that had gotten stuck on the sticky traps and used this as a measure of the powder's effects on flight ability. The UV-powders turned out to have no overall negative effect on survival nor flight ability.

I then developed a device that collected insects alive from the Malaise traps and painted the inside of these devices with the UV-powders. This caused the insects to passively mark themselves as they moved around inside the collecting apparatus. I took the marked insects to an experimental site where I released them inside a network of new Malaise traps. I then collected samples from these traps on a daily basis and continued to release marked insects simultaneously. By photographing all individuals before release, I knew how many I released. When going through the samples under a UV light, I could spot marked individuals and know how many were recaught. This method and the device developed were effective, with a recapture rate of 4.5% for wild individuals. This number is in line with studies using other, more time-consuming methods of mark-recapture for insects. My method is thus an improvement to these methods, as it requires much less physical labor. However, lab-reared insects released had no recaptures. While the reason for this is unknown, I believe the behavior of these insects differ from the behavior of wild, natural insects, making the lab-reared insects much less useful for studies such as this.

To understand the insects' activity patterns, I set up 24 Malaise traps within a 60x60 meter square. I emptied the traps every second hour between 06:00-22:00, with one additional emptying at 02:00, for five consecutive days. I also recorded temperature, wind speed, relative humidity, and cloud cover at each visit. I then related insect activity to weather by examining how the number of insects caught, divided into different taxonomic groups, changed with weather conditions. I also measured the insects' wing- and body areas to see if these traits were connected to activity patterns. Temperature had the strongest effect on the number of insects caught in the traps. Different insect groups reacted differently to temperature, however almost all of them increased in activity with increased temperature. Wind speed and cloud cover lowered activity for all groups. Relative humidity had no effect on insect activity. With increased temperature and wind speed, insects with small wings in relation to their body size became more dominant in the traps. Body area was not related to any weather variables.

In summarize, in this study I wanted to see how activity affected detectability of insects in Malaise traps and develop a method to collect data for future studies on how movement patterns of insects affect their detectability in Malaise traps. To do this, I developed a device for marking many small insects with harmless UV-powders. I released these insects and recaught them in the wild, proving this method useful for future studies of insects' movement patterns in relation to Malaise traps. I then looked at the insects' activity patterns in relation to weather variables. Temperature had the greatest effect on activity and thus detectability, increasing abundances for almost all groups. Increased wind speed and cloud cover both lowered detectability of all groups. Temperature and wind speed also changed the composition of the samples as insects with small wings in comparison to body size were more common at higher temperatures and wind speeds.

In conclusion, a Malaise trap will catch a different number of individuals depending on weather. However, the ratios between the most common groups will stay close to unchanged. Since other common groups that were not examined here are of similar sizes and are closely related to the studies insects, there is reason to believe that this pattern would also hold true for these groups. The results should also be applicable to different habitats and regions of the world, although more research concerning local effects on insect activity and detectability in relation to weather is needed to say anything conclusive. In addition, future studies on

movement patterns of these insects will be greatly facilitated by the mark-recapture method developed here.

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