Master's Thesis

Comparison of the flight metabolic rate and reproductive success of meadow brown (*Maniola jurtina*) butterfly between two landscape types

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Fragmentation of landscapes is known to affect dispersal and survival of different organisms. With increase in farmland, there also a need to know how these organisms, especially butterflies adapt to this changing landscape. In butterflies, flying is the way to disperse in the fragmented landscape, but it is highly energy demanding. When energy is demanded for other functions, there is a chance that there would be a trade-off towards other traits, like in this case a possible trade-off between fecundity and flight metabolic rate. To answers these questions, I measured flight metabolic rate (FMR) of adult meadow brown (*Maniola jurtina*) butterflies, a grassland butterfly, from both intensive agricultural landscapes and meadow landscapes closer to semi-natural grasslands. FMR was calculated based on the CO_2 produced during a 10 min flight. Some of the females were then used to determine reproductive success by counting the eggs laid during the given 7 days after the FMR measurements and by the percentage of hatched caterpillars from said eggs. It was found that there was a significant difference in the maximum metabolic rate, so the peak of the CO_2, being higher in individuals from intensive agricultural landscapes. But with FMR (total CO_2 production) there was no difference between the landscapes. There was also a significant difference in both in the number of laid eggs and in the hatching percentage between the two landscape types, both in the favour of intensive agricultural landscape individuals. These results provide more information on the possible adaptation of the intensive agricultural landscape individuals, these females being better at dispersing and with higher reproductive success.

Palttala, Jenna	Tummahäränsilmän (*Maniola jurtina*) lentometabolian ja lisääntymismenestyksen vertailu kahdessa erilaisessa maisemakuvassa.
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Elinympäristöjen pirstoutumisen tiedetään vaikuttavan joidenkin eliöiden levittäytymiseen ja selviytymiseen. Maatalousalueiden lisääntyessä, tarvitaan myös enemmän tietoa siitä, miten nämä eliöt, erityisesti perhoset sopeutuvat tähän muuttuvaan ympäristöön. Perhosilla, tässä pirstoutuneessa maisemamosaiikissa liikkuminen tapahtuu lentämällä, mutta se on todella energiaa kuluttava toiminta. Kun energiasta iso osa käytetään yhteen toimintoon, on aina mahdollisuus, että se tapahtuu jonkin toisen ominaisuuden kustannuksella, niin kuin esimerkiksi lisääntymismenestyksen ja lentoaineenvaihdunnan välillä on esitetty tapahtuvan. Vastatakseni näihin ongelmiin, mittasin lentoaineenvaihdunnan (FMR) aikuisilta tummahäränsilmä (*Maniola jurtina)* perhosilta, jotka ovat niittyalueilla esiintyviä perhosia. Keräsimme yksilöitä intensiivisen maatalousalueen ympäristöstä ja niittymäisen alueen ympäristöstä. Lentometabolia arvioitiin 10 min lennon aikana tuotetusta hiilidioksidin määrästä. Osalla naaraista myös toteutettiin lisääntymismenestyskoe, jossa niiden 7 päivän aikana tuottamat munat laskettiin ja näistä kuoriutuneiden toukkien kuoriutumisprosentti laskettiin. Selvisi, että näiden kahden ympäristön yksilöiden välillä, maatalousalueen yksilöiden maksimimetabolia (MMR) oli korkeampi kuin niittymäisten alueiden yksilöiden. Hiilidioksidin kokonaistuotannossa, vastaavaa eroa ei havaittu. Myös munien määrässä ja toukkien kuoriutumisprosenteissa oli tilastollisesti merkitsevä ero, maatalousalueen yksilöt tuottivat enemmän munia ja omasivat korkeamman kuoriutumisprosentin. Nämä tulokset kertovat meille lisää yksilöiden mahdollisesta sopeutumisesta muuttuviin ympäristöoloihin ja siitä, miten ne hyvin ne kykenevät liikkumaan pirstoutuneessa elinympäristössä.

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TERMS AND ABBREVIATIONS

Terms

Abbreviations

FMR	flight metabolic rate
RMR	resting metabolic rate
MMR	maximum metabolic rate

1   INTRODUCTION

Increase in urban and agricultural building and intensification of agricultural systems has led to increased fragmentation of the landscape, forcing species to either adapt, disperse to other areas, or decrease in numbers (Van Swaay et al. 2006). Species in modified landscapes often must disperse to new areas and move even longer distances, and this might be difficult for smaller species like butterflies, that can't necessarily fly long distances. Butterflies' ability to fly can be measured, for example by using metabolic rates. But when more resources are allocated to for example flying, does it happen on the expense of other traits? This has been a subject of interest in life-history ecology when resources are limited and allocated among different traits. In butterflies the effect of flight metabolic rate to life history traits has been one of these interests. In this thesis I aim to give more information on the flight ability and fecundity of the vulnerable meadow brown (*Maniola jurtina)* butterfly and consider whether these traits enable the species to cope with changes in imposed by agricultural and meadow landscape environments.

1.1   Butterflies as model organisms

There is estimated to be around 2600 butterfly species in Finland which of around 1000 can be categorised as *macrolepidoptera* and around 1600 as *microlepidoptera.* Number has grown, because of new species expanding the range here (Nupponen et al. 2019, Suomen Perhosutkijain Seura 2024). For the Red List of Finnish Species (2019) a total of 2 362 butterfly taxa were evaluated. 20 were regionally extinct, 421 vulnerable and endangered, 261 near threat and 14 were data deficient. Majority of the endangered and vulnerable species (41%) is found on semi-natural grasslands and other cultural habitats (Nupponen et al. 2019). With the rapid decrease of semi-natural grasslands, the number of red-listed species living primary and secondary there has therefor increased. Main reason for the decrease of these habitats has been the overgrowing of them, this is because of the decreasing in the traditional ways of managing the areas by mowing and animal grazing, but also because of decreasing numbers in farms, regrowth of forest and the changes in the use of fertilizers and grazings. For the butterflies found in these habitats 78.5% of the species has overgrowing of habitats as a main reason for declining numbers (Hyvärinen et al. 2019).

There has been a declining trend in the number of butterfly species in Finland in the years 1999-2021 (Heliölä et al. 2022). Similar observations have been done across Europe, especially in dry grasslands, sclerophyllous scrub, and heath (Van Swayy et al. 2006). Most threatened species were found on mesophile grasslands. The trend was again declining, even though generalist species have not been declining in numbers as much as the specialist species. Agricultural change is the main reason for this decline, affecting almost 90% of the species, by

turning unimproved grasslands to single species crops with fertilizers, making them less suitable for some butterflies. All the same the abandonment of agricultural land and the change in the management of biotopes is thought to affect 65% of the threatened species (Van Swaay et al. 2006).

1.2   Land use

Land use is the modification and management of natural environments suitable to human use, it includes buildings, residential areas, and city regions, but also forestry and agriculture (Finland's environmental administration 2024). Land use has a big role in shaping ecosystems and biodiversity, and it affects a variety of species, butterflies being one (Habel et al. 2021). In Finland 4.4% of the land surface is build land and 9% agricultural land (Statistics Finland 2014). Agricultural land contains everything from fields to farms and gardens and areas surrounding the fields from water sources and roads. The landscape includes meadows, pastures, and old cattle grazing grounds also known as semi-natural grasslands (Tiainen et al. 2004). Fields make up about 7% of the land area in Finland (Finnish Environment Institute 2023). Even though the number of farmers has been going down, the size of the farming lands has increased from 52 hectares to 53 hectares per farmer from 2022 to 2023, when during traditional agriculture in around 1880 the size of farming lands was 0.4 hectares (Tiainen 2004, Natural Resources Institute Finland 2023). This increase in farm sizes also increases fragmentation since forest patches and banks with suitable habitats for butterflies are now even further away from each other.

Agricultural land use has been with us for a very long time, and it has had great impact on the habitats surrounding them, especially with the increase in intensive agricultural land use, with heavy machinery and increase use of fertilizers, pesticides and with exceedingly homogenous crops. We can still see the effects of earlier ways of farming, especially the slash and burn method, where the forest was first cut down from a designated, small area and then burned. This way the nutrients were available to the crops, farmed on the spot. This affected the tree species composition in the forest patch used for farming, and sometimes there was no regrowth of the forest, but the area was left to be used as grazing ground, or for growing hay for livestock (Tiainen 2004). These human influenced landscape types are called semi-natural grasslands and wooded pastures. These were taken care of by the animals grazing on them and by mowing. Since this type of farming is not common anymore, the number of semi-natural grasslands has declined during the 1900s by 99 % (Hyvärinen et al. 2019). These semi-natural grasslands are important and can have a positive effect on biodiversity. While fields with the majority of plants being one crop can be challenging environments for any species, especially to flower visiting insects like butterflies. This is because there is no abundance of flowering, nectar plants in the crops, and it has been shown that the insect abundance in general is highly correlated with floral nectar sources (Wallisdevries et al. 2012). While plants that

are considered weeds in fields like *Cirsium* and *Centaurea* are one of the most popular with butterflies according to Wallisdevries et al. (2012) and removing them also affects the numbers of butterflies visiting the site. In this thesis I also focused on the intense agricultural landscapes and meadow landscapes closer to the semi-natural grasslands, which are still preserved in Åland islands compared to the mainland Finland (Ahonen et al. 2018).

Fragmentation caused by increased land use has led to less suitable habitats. Fragmentation includes both the habitat loss, meaning the reduction in the size of the area and separation of habitat patches (Fahrig 2003). Fragmentation can however also be seen in the increase of habitat patches, decrease in the size of those patches and in the increased isolation of said patches (Fahrig 2003). Loss of quality in these patches decreases the populations growth rate, increases the fluctuations in the population and the risk of extinction. For butterflies one of the resources affecting the patch quality is the nectar plants available for adults to feed on. Amount of nectar plants also affects the movement of the butterflies and increases immigration, while decreasing emigration (Hanski 2005).

One of the negative effects of fragmentation is negative edge-effect, where the more edge the patch has, more changes there is for species to leave the patch and move to the matrix (area with no habitat). Time spent in the matrix has been shown to reduce reproductive rate and increase mortality rate (Fahrig 2003). Fragmentation of habitats can have multiple effects regardless of if it's the loss of quality or quantity, decreasing the population size, increasing the edge-effect, and reducing genetic variation. Or the loss of connectivity and continuity reducing immigration even more, isolating the patches from each other even further (Hanski 2005). With the increased fragmentation the population slowly turns into metapopulation and dispersal between these patches can be important to the survival of these metapopulations. Dispersal has also been noticed to be affected by the fragmentation of the landscape, since the cost to disperse is higher with longer distances and increases mortality (Schtickzelle et al. 2006).

1.3   Dispersal and flight

Flying has been a significant event in the evolution of insects giving them an improvement in finding mates, food, escaping predators and dispersing to new areas and is therefore used in almost all adult functions (Gullan and Granston 2014). The ability to fly is thought to have evolved around 300 million years ago, oldest winged insect being *Delitzchala bitterfeldensis* that lived 325 million years ago (Ross 2017). In modern insects only the adults have fully functional wings, even though some of the nymphs have visible wing buds, they are not functional. To fly insects need to overcome the drag and the force of gravity. In gliding flight overcoming these forces comes with the help of relative winds, that are passive air movements, this is done by changing the angle of the forewings. For examples dragonflies (Odonata) and grasshoppers (Orthoptera) glide a lot (Gullan and Granston 2014). In flying insects the muscles powering the flight are arranged

either as direct flight muscles or as indirect system. In the direct system, the muscles connect straight to the wings, where the upward stroke of the wing is produced by contraction of the muscle attached to the wing base inside the pivotal point and downward motion by contracting the muscle going from the sternum to wing base, outside of the pivotal point. The indirect flight muscles on the other hand are connected to the tergum and sternum, which when contracting the muscles at the base of the wing bending it down, causing the tips of the wings to rise (Gullan and Granston 2014).

Dispersal is in its simplest form, one way movement of individuals between natal places. Dispersal can occur from place of birth to breeding grounds, but it also might happen after food sources (Matthysen 2012). Dispersal could ultimately also lead to gene flow if breeding is possible in the new area. Breeding dispersal is movement between two places of reproduction, these two methods are the most likely result to gene flow (Clobert et al. 2001, Starrfel and Kokko 2012). Dispersal can be a response to changing environment and to its large-scale modifications, when nothing else can be done, or the change is too fast for individuals to adapt. But at the same time, it helps species to spread to new locations (Clobert et al. 2012). Dispersal can be seen as a three-stage movement forming from emigration, transfer, and immigration, this however is not always the case like with nomadic birds (moving based on the abundance of food sources in the area), or primates moving to other social groups after maturity (Matthysen 2012). Dispersal mechanisms are either active or passive dispersal, active being where the individual is in control of its own movement, following cues from social and physical environment. Passive dispersal is when the individual does not have any control over the movement happening, and it usually happens due to external forces (Matthysen 2012).

Dispersal has a lot of possible consequences that can be thought as costs and benefits of dispersal, these are also the evolutionary forces shaping the dispersal patterns. First one is the decrease in kin competition (and competition in general) and inbreeding when some disperse to other area. This comes however with a cost of kin cooperation and the possible costs of outbreeding (Lambin et al. 2001, Starrfel and Kokko 2012). Predation and parasitism are also thought to be one of the forces guiding dispersal, there is however very little studies done in interspecific competition compared to intraspecific competition. Dispersal happening because of parasites or predation is the avoidance of them (Weisser et al. 2001)

Flying is on way to disperse but takes a lot of energy. Butterflies for example use energy stored in larval stage as abdominal lipids, but also get some carbohydrates from adult foraging (Lebeau et al. 2016). In experiments done on the effect of nectar quality and quantity to a flight performance in meadow brown butterflies it was found that there was a significant difference in the flight performance between the different nectar supply (Lebeau et al. 2016). In honeybees (*Apis mellifera*) flight metabolic rate (FMR) have been shown to vary greatly with temperature and air pressure, but also within individuals, depending on their role in the hive. Foragers, flying in low-density air or having

a heavy load had higher FMR than those flying in higher temperatures (Harrison and Fewell 2002).

Measuring dispersal in butterflies can be done with multiple ways. Including for example mark-release-recapture (MRR). Where the butterflies are captured from one location, marked, and released back. Then after a certain time butterflies are captured again, and a flown distance can be calculated (Stevens et al. 2010). Measuring of FMR is also a way to estimate individuals flight distance energetics and ability, and therefore dispersal abilities of individuals. This is done by simply measuring the total amount of CO_2 produced by the individual, during a certain time limit (Niitepõld et al. 2009a). FMR is the rate of which an organism consumes energy during flight. One way also to measure dispersal is to use body or wing size as a proxy. With longer wingspan and larger wing area insects are expected to disperse over greater distances (Dudley 2000, Roff 1990).

1.4   Fecundity related trade-offs

Trade-off happens, when one trait is increasing in performance with a cost of another trait. Trade-offs have been studied a lot and many of these studies are done regarding reproduction or fecundity. FMR is hypothesized to influence for example fecundity, because flying is a high-energy activity and investing into it, meaning butterflies with higher FMR would allocate more energy to flight with the expense of reproduction. It has been noted in butterflies, for example, that fecundity rate often decreases when the life span increases, but longer life also provides a chance to lay eggs more often (Haeler et al. 2014). On *M. nurag,* fecundity decreased along with the duration of dormancy and depending on the body mass. This indicates that *M. jurtina* could be able to expand the life span as a response to environmental changes, but without it affecting the fecundity (Haeler et al. 2014). Adult body size again is dependent on the nutrition received on the larvae stage since the nutrition will be allocated during metamorphosis. This again contributes to the traits of adult life history, shown to increase survival and increased adult food intake for reproduction (Boggs et al 2005). Fecundity of the *Speyeria mormonia* females have been observed decreasing when body mass and fat content decreased. When looking at the food intake in both larval stage and in the adult stage, it was concluded that together the two food allocations affect the fecundity and survival of the individuals, having possible effect also to flight and therefor dispersal (Boggs et al. 2005). Similar results have been observed in the tropical *Bicyclus anynana,* with nutritional limitations in larval stage decreased the body mass and increased thorax ratio. This happens at the expense of reproduction since there is less space in the abdomen (Saastamoinen et al. 2010).

The trade-off between fecundity and dispersal has most often been found in wing-dimorphic insects (those that have two morphs, one with wings capable of flying and one without or with very short wings, like ants or aphids) (refs). It remains unclear whether similar trade-offs occur with monomorphic insects (with only one morph, like butterflies), even though this is often idea adopted

my scientist (Roff and Fairbairn 1991, Hanski et al. 2006). There have been observations of the opposite, that there could be a positive link between dispersal and fecundity, at least in the Glanville fritillary (*Melitaea cinxia*) *(*Hanski et al. 2006*)*. More dispersive females have been observed to lay more eggs, than females that are less dispersive. The more mobile females also had higher egg production during their lifetime than less dispersive females (Hanski et al. 2006). With unlimited food the Glanville fritillary females had increased fecundity and laid eggs earlier than the control ones. However, when 50 % food restrictions were placed, the fecundity dropped, when comparing to the unlimited food individuals, but had still higher fecundity than the control group (Niitepōld 2019).

1.5 Aims of the thesis

Aim of the thesis is to try find the effect of the place of origin, namely natural meadows vs. Agricultural landscapes, to the flight metabolic rate in the meadow brown butterfly. And to identify possible trade-offs between said FMR and reproduction of these butterflies. Flight metabolic rate can be used to study the dispersal of species, since the FMR can tell us about the dispersal ability of an individual, since it represents flight ability and is also used in this thesis for the meadow brown.

This thesis addresses the following main questions:

- Is there a difference in the flight metabolic rate between the individuals from different landscape types?
- Does the flight metabolic rate affect the fecundity of the meadow brown?
- Do the amount of eggs produced by individuals from different landscape types differ?
- Does the eggs from different landscape types, hatch in different rates?

I hypothesize that individuals from agricultural landscape types will have higher FMR. I also hypothesize that individuals from meadow landscape types will produce more eggs. There could be possible trade-off between the FMR and fecundity in agricultural landscape type individuals, decreasing the number of eggs produced, while increasing the FMR. There will most likely also be a difference in the hatching rate of the caterpillar since individuals from agricultural landscape types would most likely have higher survival rate.

2 MATERIALS AND METHODS

2.1 Study species

Study species used is the meadow brown butterfly (*Maniola jurtina*). This butterfly is classified as vulnerable in Finland because of its limited habitat availability, due to overgrowing of meadows and open habitats (Punainen kirja 2019). Sightings of the meadow brown have increased in recent years (Suomen lajitietokeskus 2023). In Finland it is found only in the Åland islands and in the southwest of Finland, even though in Europe it is one of the most common butterflies (Suomen lajitietokeskus 2023). Suitable habitats for the meadow brown butterfly are meadows and fields since its caterpillars use multiple different kinds of grasses (*Poaceae)* as their food source. Adults have been shown to prefer nectar from C*entauria jaucea* as their food source *(*Lebeau et al. 2016).

In meadow brown, females are generally larger than males and they are easy to distinguish from each other. On top of the size difference, the males are generally darker, especially in the time just after eclosion. Both sexes have an eyespot on their upper wing, but in female the spot is larger and has a bigger area of orange surrounding it, that can sometimes cover majority of the upper wing. In males these eyespots are smaller and have only a small ring of orange around them. Males also have small eyespots on the underside of their lower wing, while females have a lighter band (Fig. 1) (Butterfly conservation Yorkshire 2020, Luontoportti 2021, Suomen perhoset 2022). Meadow brown is an univoltine butterfly, meaning it has only one brood of off-spring and overwinters as a caterpillar (Brakefield 1982). Flight time in northern Europe is from end of June/early July until August/September, depending on the season, with male emerging first and females about a week after (Haahtela et al. 2019, Suomen lajitietokeskus 2023). Eggs are laid one at a time on grasses, sometimes even on flight.

Figure. 1. On the left a male meadow brown and on the right a female (Malinen 2023).

2.2 Study area

Åland islands is an area in the southwest of Finland, consisting of 6 700 islands. Because of its southern position, Åland has many deciduous trees. Åland is also known for its meadows, forest pastures and leas, that have been formed by the grazing of farm animals like cows and lambs (Kontula and Raunio 2018).

Butterflies were collected from two different types of landscapes in Åland: those dominated by meadow and those dominated by agricultural land. The study sites were chosen based on aerial images and on-site visits, after which the land use was quantified based on the CORINE land use data from 2018. In the end we chose six locations, three agricultural landscapes and three meadow landscapes across the Åland islands (Appendix 1). Description and the number of collected butterflies from each site are seen in Table 1.

TABLE 1.   Locations and descriptions of study sites. Also number of butterflies collected from each site. A being agricultural landscape type and M meadow landscape type.

Location	Landscape type	Description	Number of collected butterflies
Haga, Saltvik	A	Large (locally), continuous agricultural landscape with some small fragments of natural landscape in between the fields. Hay and cereals being the major crops, with occasional rapeseed field and apple orchard.	17
Kyrkoby, Eckerö	A	Relatively large agricultural area and continuous agricultural landscape with some natural landscape and fragments of small horse paddock. In this location potatoes, cereal crops and hay are the main crops.	20
Gottby, Jomala	A	Agricultural landscape with cultivated fields (mainly potatoes, hay and cereal crops), semi-natural cattle paddocks and natural areas (mainly forest and some shrubs) between the cultivated fields.	13
Bomarsund, Sund	M	Meadow area with semi-natural meadows and some semi-open woodland that are maintained by cutting and/or grazing (by sheep).	19
Storby, Eckerö	M	Natural meadow consisting mainly of dry rocky coastal meadows with some shrubs.	13
Husö, Finström	M	Natural meadow with small-scale hay fields maintained by cutting and/or sheep/cattle grazing. Fragmented by patches of forest/woodland.	12

2.2.1 Butterfly collection and handling

At first a total of 27 female meadow browns were captured and used to determine the most suitable temperature at which to conduct the final flight metabolic rate experiment. We decided to use female butterflies because we were also going to test the reproductive success of these butterflies. For the main FMR measurements 67 butterflies were collected from Bomarsund, Haga, Eckerö (two areas), Gottby, Husö in Åland. Of these Haga, Gottby and one of the Eckerö areas were agricultural land and Bomarsund, Husö and the other Eckerö area were meadow areas (Appendix 1).

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Butterflies (N= 94) were captured using an insect net and transferred to a small cylinder-shaped cloth cage (10 x 25 cm) for transportation, where from every location the butterflies were placed in the same cage. These cages were then put into a cooler to calm the butterflies and to make it easier to handle them. After capturing the butterflies, one cage at a time was being handled indoors to prevent the butterflies from escaping, one butterfly at a time was taken out and an ID number (a running number, starting from 1) was marked to the underside of the right lower wing (Fig. 2). After marking the butterfly, they were put to a bigger cage (38 x 38 x 58 cm) with some honey water (1:10) for food. When marking a butterfly, the ID, where it was captured, the date and the landscape type were written down. This way it was known which butterfly came from which area. This was repeated to all cages caught that day. The butterflies were allowed to feed and move freely within the cage until the same evening, after which they were transferred back to the smaller cages and a cooler box that was left outside to wait for the measurements the next day. The food was removed at the same time since the butterflies were inactive and not feeding during the night (Table 1).

Figure. 2. Numbers were marked to the underside of hindwing on the right side.

2.3 Flight metabolic rate measurements

The flight metabolic rate measurements (FMR) were conducted within a semi-enclosed chamber consisting of plywood, plastic cover, UV-light and portable heaters, so that the ambient temperature was controllable (with small variation), either to increase the temperature by increasing the heat in the heaters and

closing the plastic cover, or to decrease the temperature by decreasing the heat and lifting the plastic cover.

Inside the chamber the temperature was monitored using Sable Systems NTC thermistor (Sable systems, Las Vegas, NV, USA). Flight metabolic rate measurements were done using open-flow respirometry. Drierite (W.A. Hammond, Xenia, OH, USA) was used to eliminate water vapor from the air entering the respirometer, and using Medisorb (GE Healthcare, UK), the CO_2 was scrubbed. The 1 L respirometer was supplied with air at a flow rate of 1.0 L min^{-1}, with a Sable Systems SS3 subsampler. Following this, the air underwent a drying process with magnesium perchlorate (Alfa Aesar, Karlsruhe, Germany) before going into the CO_2 analyser (Li-Cor 6251, Li-Cor Biosciences, Lincoln, NE, USA). Data of the flow rate, respirometer temperature, CO_2 concentration, and Li-Cor temperature were converted into digital format through a Sable Systems UI-2 interface and subsequently logged using a laptop PC equipped with Sable Systems' ExpeData software (Niitepõld et al. 2009a).

Figure. 3. Setting inside the plywood box.

Butterflies were taken to the lab 1 h prior to the measurements, to warm them, but kept under cloth to prevent flying. One butterfly at a time was place in the 1 L chamber, covered with a cloth to calm down and attached to the respirometer to remove CO_2 from the chamber at the same time temperature was adjusted to desired. When the CO_2 level had reached a steady baseline, the cloth was removed and if the butterfly started to fly, it was left to fly on its own, but if not, the chamber was shaken and tapped lightly to encourage the butterfly to fly. Each butterfly was flown for 10 min and then set back under the cloth to calm and the CO_2 level to go back to the steady baseline. During the measurement the total volume of CO_2 and the highest rate of CO_2 was calculated. After CO_2 level was

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steady, the measurement was stopped and save to the computer (Niitepõld et al. 2009a). After each measurements the respirometer was calibrated. From the butterflies a gene sample was taken, by removing one of the legs (preferably one of the atrophied front legs) of the butterfly (and the whole butterfly if it died), also the weight and forewing length was measured.

First task was to determine the most suitable flight temperature for the Meadow brown. This was done by assessing FMR at five different temperature groups 23—24 °C, 25—26 °C, 27—28 °C, 29—30 °C and 31—32 °C. In each temperature groups we tested five different butterfly individuals. For each temperature measurements the butterflies were flown for 10 minutes by gently shaking and/or tapping the container whenever the butterfly tried to land on the side of the chamber. There were no significant differences between the temperatures, but there was a trend which indicated a better flight performance in higher temperatures. Hence, we chose to use a temperature of 31±1 for the final FMR assessments.

2.4   Reproductive success

After FMR measurements, the butterflies (N =47) were set in the cage to feed off the honey water provided on a sponge. Next day the butterflies were set on cages with a potted plant in them, we used grasses that are known to be the food source for the caterpillars as oviposition plants. The cages were set so that they get at least a few hours of sun a day and they were misted few times a day so that the butterflies get a chance to drink, and it also prevented the cages from overheating. Each cage had a tape that had the ID, capture location and the date when the butterfly was set into the cage. Each of the butterflies were allowed to lay eggs for seven sunny days, giving them a good chance to lay eggs, since meadow browns usually lay the eggs within a few days of mating. Butterflies were fed every morning, meaning that fresh honey water was provided on a sponge.

Eggs were collected after the seven sunny days. For a few females we noticed that the nets had larger holes in them and that some of the eggs had disappeared during the night. In these cases the cages were checked every day, and any found eggs were taken out. In the cages with smaller holes, the number of eggs was checked on the evening of the 7th day, when the butterflies were already inactive and due to be released back to the wild the next day (Fig. 5). The eggs were picked up with a toothpick and/or cotton swab and set to a petri dish with a filter paper on the bottom. To the lid the ID, location, species, and date were written, and the lid was taped to the bottom to prevent the eggs from falling off in case the petri dish was dropped. The petri dishes were set to a growth cabinet, so that every egg would have the same growth environment. The cycle was set to 99, meaning the cycle would continue indefinitely, until it was stopped (Table 2).

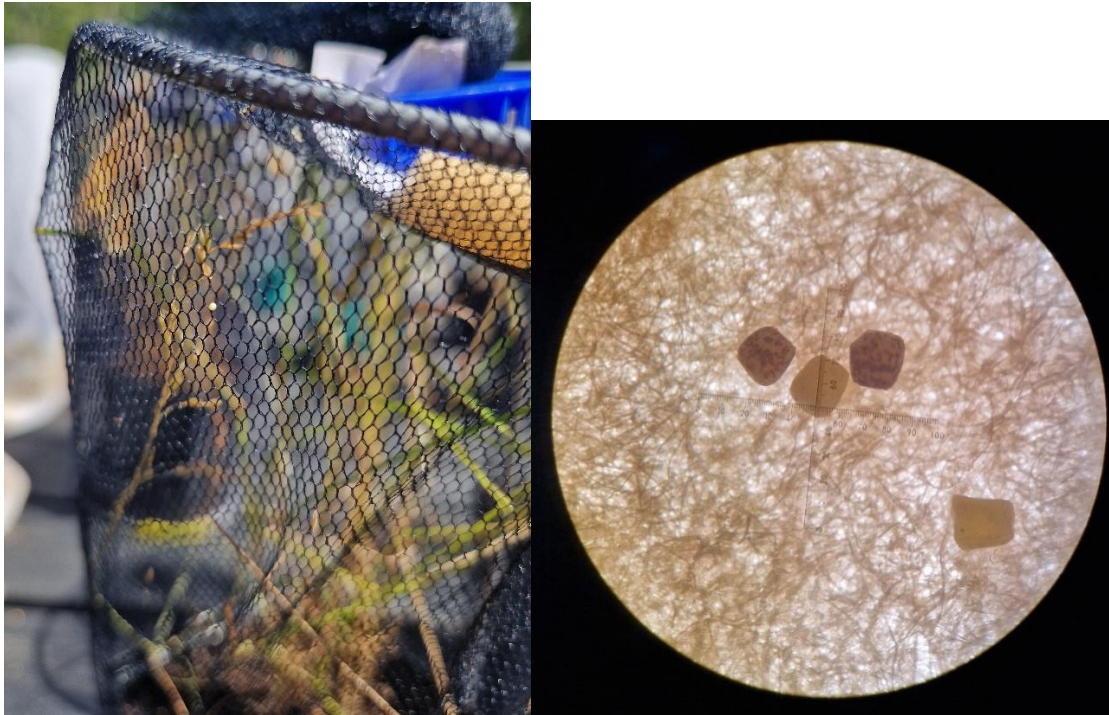Figure. 4. Some of the egg laying cages (Photo by Suvi Ikonen).

Figure. 5.   Meadow brown butterfly eggs at the side of the cage (left) and eggs under the microscope (right).

TABLE 2. Settings used in the growing cabinet. Time is set, when the wanted temperature was to begin and light is the amount of light in the cabinet, at the given moment, 5 being full light and 0 being no light.

Time	8:00	12:00	17:00	23:00
Temp (°C)	20.0	25.0	20.0	15.0
Light	5	5	5	1

After eggs were set to the growing cabinet, they were checked twice a day for caterpillars. Caterpillars were calculated each day if hatched and they were given food in form of grasses and a bit more water. It was noticed that the caterpillars were fragile and small, so they were given a couple days to grow and bulk up, so that the transfer to the jars was easier. The jars used, were plastic jars with a net lid, inside was two filter papers at the bottom. Each jar had 10 caterpillars from the same female and the ID, location and habitat of the female was written on the side of the jar. After the female had been laying eggs for seven days it was released back to the same location where it was collected.

2.5   Statistical analysis

Data was analysed in both R studio (version 2023.06.1) and Microsoft Excel (version 2311). First, values were extracted from the raw flight metabolic rate data using an R script that calculates the highest emission rate of CO_2 (peak flight metabolic rate in ml/hour), temperature at the highest peak, total volume of CO_2 during the whole measurement period, highest peak during the last 3 min and an average temperature during the last 3 min. And for the resting metabolic rate (RMR), average RMR, average temperature during resting.

In R studio, after the loop had run and all the values were calculated, the background data that contained information on the weight, location, habitat, ID, etc. of the butterflies was added. Maximum metabolic rate (peak CO_2 during the 10 min period) and total CO_2 production were corrected with individual's weight since metabolic rate often correlates with body mass. This was done by making a regression model with mass and the metabolic rate value and using the residuals from the model in the analysis. After correcting the MMR and Total CO_2, the first 27 individuals were removed from the data, since they were only used to determine the optimal flight temperature. FMR, MMR and RMR were corrected by the mass of individuals, since it is known and noted with this data that the mass of the butterflies correlated with the metabolic rates.

To analyse the effect of different variables to the CO_2 production, a linear mixed model was used (LMM). Containing corrected total CO_2 or corrected MMR as a dependable variable and different combination of fixed variables consisting of landscape type, egg production, temperature at the time of maximum metabolic rate and time of measurement, counted as minutes from 08 in the morning of the measurement, as a random variable in each model was location and measurer variables. Best model was selected based on the lowest

AIC value and in the end only landscape type was chosen as a fixed variable (Appendix 2). For the reproductive success generalized linear model (GLM) was used with Poisson family for the egg production and binomial family for the caterpillar hatching rate.

3 RESULTS

3.1 Flight metabolic rate

Individuals from agricultural landscape had greater FMR than individuals from meadow landscape types, difference being statistically significant (Fig 6 A; LMM, t_{71} = -2.08, p = 0.04). Total CO_2 production had no significant difference (Fig. 6 B; LMM, $t_{4.346}$ = -1.64, p = 0.17). There was no significant difference in resting metabolic rate between females originating from different landscape types (Fig 6 C; LMM, p > 0.05) (Fig. 6).
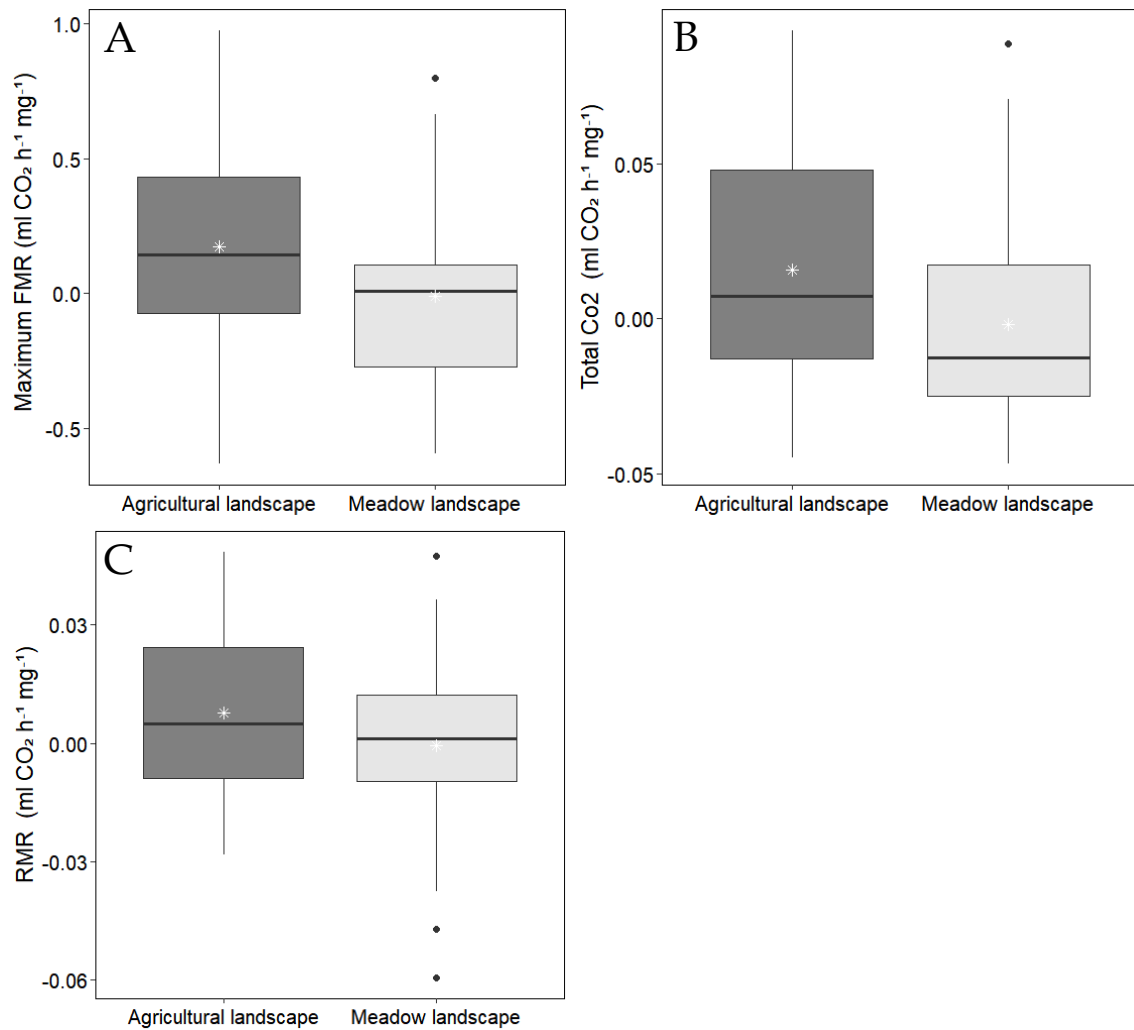
Figure. 6.   The maximum flight metabolic rate of the individuals during the 10 min flight (A), total CO_2 production during the same time (B) and rest metabolic rate (RMR) (C) of the meadow brown butterfly (N = 74) from both landscapes of origin. Values on the y-axis are mass corrected.

3.2 Reproductive success

There was a slight negative correlation between egg production and total CO_2 in both landscape types, but neither was statistically significant (Fig. 7 A; Agricultural: r (13) = -0.29, p = 0.29 and Meadow: r (12) = -0.04, p= 0.88). Correlation in general without the separated land use types was not statistically significant either (p = 0.90). Results were similar between egg production and MMR, neither was statistically significant (Fig. 7 B; Agricultural: r (13) = -0.32, p = 0.24, And Meadow: r (12) = 0.24, p = 0.42). Landscape type had no effect if the female laid eggs or not (p = 0.88) but the difference in the number of laid eggs

differed significantly between females from the two landscape types (Fig 8 A; GLM, Estimate = -0.14, SE = 0.06, z = -2.40, p < 0.001): females from agricultural landscape type laid more eggs in total than those from meadow landscape type. From the total of 47 females set to lay eggs, 29 laid eggs. Of the eggs laid, by females originating from agricultural landscapes the percentage of hatched larvae was greater (80%) than in meadow habitats (68%) and the difference was statistically significant (Fig 8 B; GLM, Estimate = -0.63, SE = 0.13, z = -4.70, p < 0.001). While from the eggs laid by agricultural individuals, each female had at least one caterpillar hatched, in meadow individuals there was few females that had no caterpillars hatched from the eggs produced.
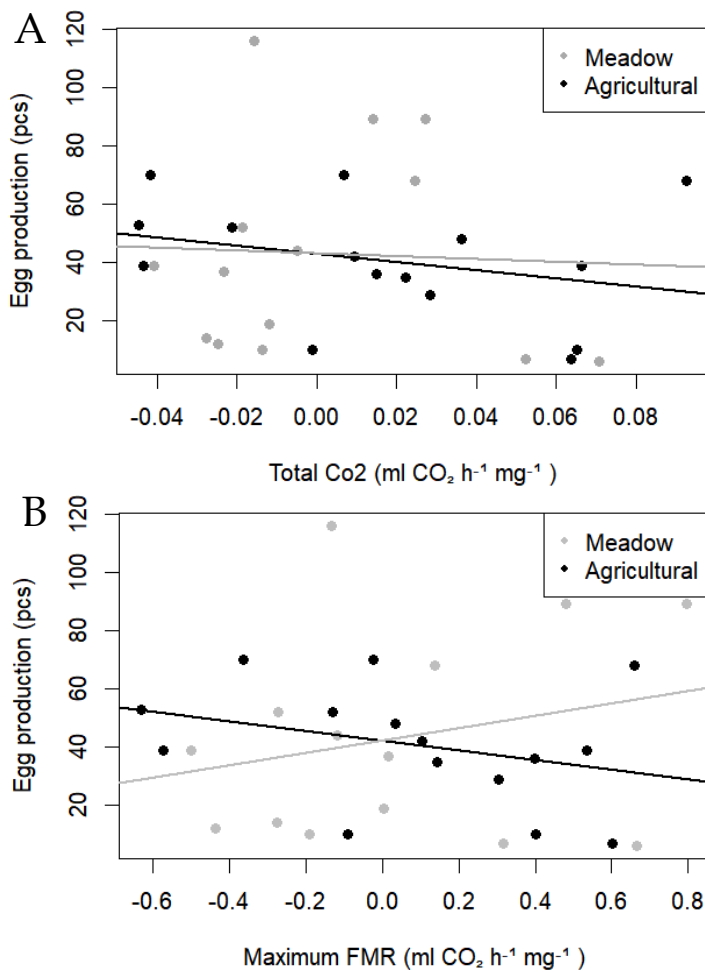

Figure. 7.   Correlation between egg production and Total CO_2 (A) and egg production and MMR (B) of individuals from both landscape types. Values on x-axis are mass corrected.
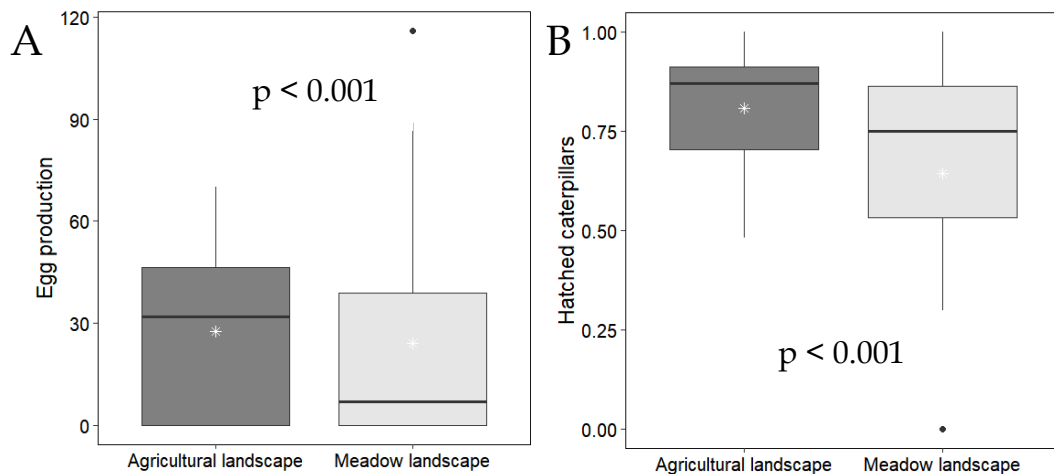
Figure. 8. Egg production per landscape type (A) and the percentage of hatched caterpillars per landscape type (B).

4 DISCUSSION

With the increasing amount of building and agricultural landscapes, the space for other species is getting smaller and more fragmented. This can have significant effects to the populations survival and compositions in the continuous landscape of crops, buildings and roads. My aim was to find if there was a difference in the flight metabolic rate of the meadow brown butterfly individuals originating from intensive agricultural landscape and from more natural meadow landscape types. I was also interested in the reproductive success of these butterflies and whether there is a trade-off between dispersal ability and reproductive performance. What was found was that there was a significant difference in the maximum metabolic rate (MMR), it being higher in agricultural landscape type individuals. Similarly agricultural landscape individuals laid more eggs and the hatching percentage from these eggs was also greater compared to the meadow landscape type individuals.

4.1 Impact of landscape type on dispersal ability and potential trade-off between dispersal and reproductive performance

Results showed that females from intensive agricultural landscape types were better at dispersing. Differences in the flight metabolic rate come from the MMR (maximum metabolic rate), being higher in agricultural landscape type individuals, suggesting that these butterflies must make longer bursts of flight. This can be supported by comparing the landscape types themselves, in the intensive agricultural landscape, suitable nectar plants for adults and food plants

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for caterpillars are scarcer, having the butterflies fly longer distances. With FMR (the total CO_2 production) there was no significant difference between the individuals from different landscape types. This is partly in support of my hypothesis, since the MMR was higher in intensive agricultural landscape individuals, while the FMR (total CO_2) was not. Possible explanations for the results could be that because female meadow browns usually fly mainly to feed and lay eggs. This could explain both results in MMR and FMR. In the wild females move from one nectar plant to another longer distances in agricultural landscape type, but in total females generally fly less, resulting no difference in FMR (total CO_2) (Brakefield 1982). Place of origin also didn't affect the FMR when comparing in different nectar quality and quantity treatments. Intensively manage, nectar-poor agricultural landscape individuals did however show stronger flight performance, indicating higher MMR (Lebeau et al. 2016). Similarly intensive agricultural meadow brown females, have been able to buffer the loss of body mass when a nectar supply is available, also having greater survival rate than individuals from extensively managed grasslands (Lebeau et al. 2018). At the same time, fecundity was not affected by the place of origin, which is different form my observations.

In regards the reproductive success, the landscape type where females were collected from did not influence the willingness of the females to lay eggs, but it did have a significant effect on the number of eggs they laid. Again the individuals originating from agricultural landscape types, produced more eggs and had more consistent amounts of eggs between females. Individuals from meadow landscape types on the other hand, produced eggs a bit more inconsistently, with few females producing great number of eggs, while some produced none. Agricultural landscape individuals also had a higher hatching percentage, having more caterpillars hatched from the eggs laid and every individual had at least one caterpillar hatched, while meadow landscape type individuals had a few with no caterpillars' hatch. So from the reproductive success point of view, individuals from agricultural landscape types seem to be able to reproduce more efficiently. Better reproductive success in agricultural landscape type individuals could be an adaptation to the landscape, having fewer hiding places and suitable plants scarce, meaning that having more offspring and higher hatching percentage would give a change to at least some of the offspring to survive to adulthood. While meadow landscape type individuals would have more hiding places and suitable plants for eggs and caterpillars, there being no need to invest in the number of offspring. It is also possible that only "good" individuals are the ones surviving and could even disperse to these more challenging landscapes, resulting in these findings. But to study this with the current data is not possible and we would need data possibly from adult survival and caterpillar survival to identify what could cause the better reproductive success in the agricultural landscape type individuals. Possible explanations could be, because females originating from agricultural landscape type have been observed having a better buffering, when it comes to the changing availability in nectar, whereas meadow landscape type females do

not buffer the change as well (Lebeau 2015). There is also always the possibility that females captured, had already laid the eggs, or weren't mated yet, even though we tried to capture the butterflies as early as possible, but it is never certain when the individuals have emerged when capturing wild individuals.

Contrary to my hypothesis, there was no observed trade-off between MMR/FMR and reproductive success in the meadow brown butterfly. In the FMR the correlations with egg production were slightly negative in both landscape types but neither was significant. In the MMR correlation with egg production, again neither landscape type correlation was significant. This time the correlation in meadow landscape type was positive, which could indicate that individuals in meadow landscape type having higher MMR would also produce more eggs. This was however just an insignificant trend, so there is no way of saying if this is the case. Positive associations between FMR and fecundity have been observed at least in Glanville fritillary (*Melitae cinxia)* where more dispersive females were found to also produce more eggs than less-dispersive females (Hanski et al. 2006).

4.2   Limitations and future study directions

The females were collected from wild for the measurement. Meaning there is no knowledge of their age, the exact location of origin, or the conditions affecting the place of origin. In the end it is difficult to say whether the differences were from genetic factors or from environmental factors, or even what would be because of the maternal effect.

The subject itself is interesting and something worth looking more into and broadening the data set. Like mentioned earlier data from the survival of adults and caterpillars, would be useful in identifying the possible survival factor to the reproductive success. Other thing would be to do flight distance observations on the field, like using a harmonic radar giving us more information about the relationship between FMR and flying distance and performance in general (Niitepõld 2009b). There could have possibly been more individuals from the two landscape types, to simply give us more data. It would also be interesting to compare both male and female individuals, to see if there is a difference in FMR, like suggested by Brakefield (1982), because of the different activities of the sexes.

4.3   Conclusion

In conclusion, there was a difference in the dispersal between the individuals originating from different landscape types with females from agricultural landscapes having better dispersal ability, based on MMR; than those, from more 'natural' landscapes. Females from more agricultural landscapes also produced more eggs and had higher hatching percentage than meadow

landscape type individuals. This could indicate that the meadow brown butterfly is adapting to the increasing fragmentation of the landscape, indicated by the higher MMR of the females from agricultural landscape types. Additionally, understanding the mechanisms underlying these differences in metabolic rates and reproductive strategies could provide valuable insights into species adaptation to anthropogenic landscapes and aid conservation efforts.

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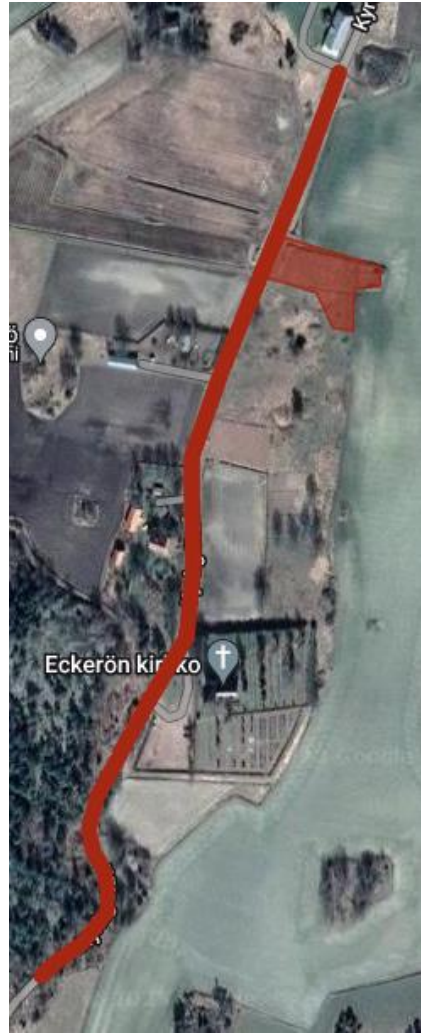
APPENDIX 1. LOCATIONS AND AREAS OF COLLECTION
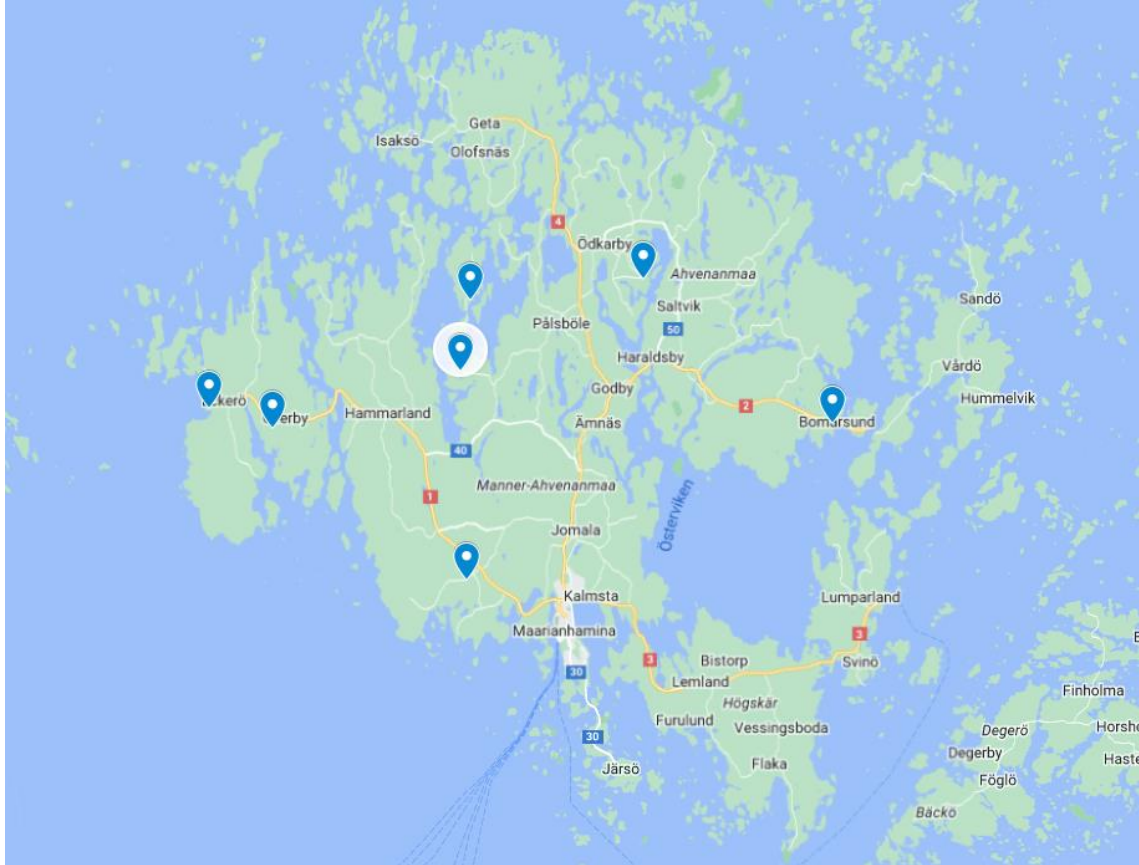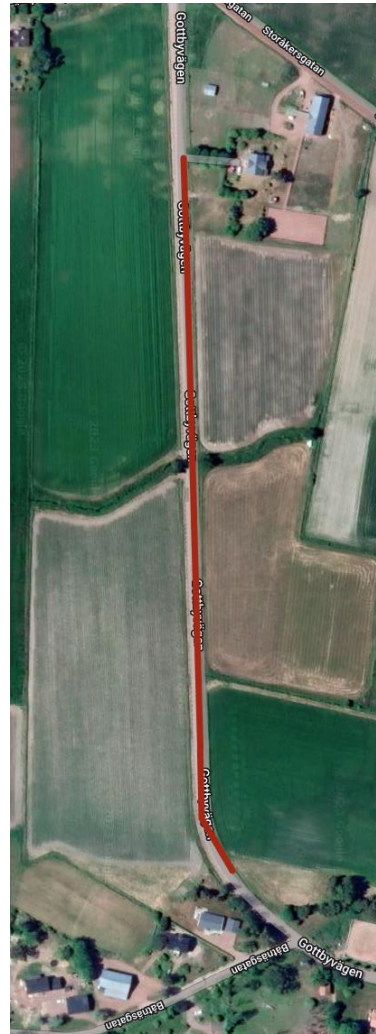
APPENDIX 2. SELECTION OF THE MODEL AND MODELS CHOSEN

Model	AIC value
lmer(Corrected.CO2 ~Habitat+Egg_prod+ MMR.Temperature+time_min+(1+ Measurer| Location),data=Final_data)	-203.66
lmer(Corrected.CO2 ~Habitat+Egg_prod+time_min+ (1+ Measurer | Location),data=Final_data)	-211.46
lmer(Corrected.CO2 ~Habitat+time_min+ (1+ Measurer | Location), data=Final_data)	-229.11
lmer(Corrected.CO2 ~Habitat+ (1+ Measurer | Location),data=Final_data)	-248.67
lmer(Corrected.MMR~Habitat+Egg_prod+MMR.Tempeture+time_min+ (1+ Measurer| Location),data=Final_data)	119.52
lmer(Corrected.MMR~Habitat+Egg_prod+time_min+ (1+ Measurer | Location),data=Final_data)	115.61
lmer(Corrected.MMR~Habitat+time_min+ (1+ Measurer | Location),data=Final_data)	102.64
lmer(Corrected.MMR~Habitat+(1+Measurer | Location),data=Final_data)	87.67

Models for the egg production and hatching percentage:

glm(Eggs~Habitat,family = poisson(),data = munat)

glm(cbind(Caterpillars,Eggs-Caterpillars)~Habitat,family = binomial(), data = munat)