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Comparing distribution of willow psyllids (*Cacopsylla spp.*) on *Salix lapponum* in 2018 with 1995 along an alpine altitudinal gradient

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Preface

This master's thesis is a part of my master's degree in natural resource management from the Norwegian University of Life Sciences (NMBU). It has given me insight into the scientific process in entomology and ecology and the work has given me an opportunity to learn first-hand from many experienced people in the field.

I would like to thank my main supervisor during this thesis work, Tone Birkemoe, for providing me with the idea for the project, connecting me with the researchers who did the previous study on psyllids in alpine Norway, and providing priceless help, tips and support throughout the work.

I would also like to thank Kari Klanderud and Ruben Erik Roos, for the guidance in ecological field work and writing, and for helping me in planning and forming the ideas along the way. Thanks to Ian Hodkinson for helping me identify the psyllid samples and for providing the foundation for this project with his numerous studies of arctic-alpine psyllid ecology. Thanks to Erika Anita Leslie for the warm welcome at Finse Research Station and letting me stay there during the field work in the summer months of 2018. Thanks also to Tone Aasbø Granerud for the laboratory guidance and providing a place to work in the insect lab at NMBU. A special thanks to Heidi Mørkhagen Granum for the collaboration throughout the thesis writing process.

Finally, a great thanks to friends and family who have been supportive and curious about my thesis and studies through the past years.

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Abstract

The psyllid (*Cacopsylla spp.*) population on *Salix lapponum* in the alpine areas of southern Norway, in an altitudinal gradient between Haugastøl and Finse, were thoroughly studied in 1995. The results were presented and published in Hill et. al. 1998. With rise in global air temperature and observed changes in insect communities over the last years, a prediction could be made that there have been changes in the psyllid population in this area from 1995 to 2018. Current findings were compared with the ones from 1995. I predicted changes to have happened in egg-laying location on the *Salix* shrubs, with a higher proportion of eggs laid on leaves rather than catkins, and changes in psyllid species composition.

I resampled the same sites along the gradient, which is located between 998 and 1222 m a.s.l. The methodology described in Hill et. al. 1998 was followed closely. Measurements were done of selected *Salix* shrubs while taking samples of leaves and catkins in early June. Enclosures were attached on leaf and catkin branches, trapping the developing psyllids inside. Further enclosures were added on leaf branches in July. All enclosures were collected in August, after any entrapped specimens would have developed into adults. The collected samples were measured and counted in the lab, followed by statistical analyses looking for trends and comparing the data with the 1995 findings.

Some differences were found in the egg placement on the shrubs, but the findings were not significant. The number of eggs on catkins had a significant relationship with catkin developmental stage and observed catkin density. Only one species of the adult psyllids were found in the resampling, and their mean abundance was significantly lower for most sites in 2018. I interpret the difference in the results as an effect of phenology having shifted from 1995 or the methods being slightly different. The temperature increase will likely also be a part of the explanation, with the 2018 summer months being abnormally warm and sunny.

Sammendrag

Bestanden av sugere (*Cacopsylla spp.*) på lappvier (*Salix lapponum*) i de fjellområdene i Sør-Norge, i en høydegradient mellom Haugastøl og Finse, ble grundig studert i 1995. Resultatene ble presentert og publisert i Hill et. al. 1998. Med økning i global lufttemperatur og observerte endringer i insektsamfunn i løpet av de siste årene, kan det antas at det har vært endringer i bestanden av sugere i dette området fra 1995 til 2018. De nylige funnene ble sammenlignet med de fra 1995. Jeg forutså at det hadde oppstått endringer i hvor eggene ble lagt på vierbuskene, med en høyere andel egg lagt på blader enn rakler, og endringer i artssammensetningen av voksne sugere.

Jeg samlet nye prøver fra de samme innsamlingsstedene langs gradienten, som ligger mellom 998 og 1222 m.o.h. Metoden beskrevet i Hill et. al. 1998 ble fulgt tett. Målinger ble gjort for utvalgte lappvierbusker og prøver av blader og rakler ble samlet inn i begynnelsen av juni. Innkapslinger ble festet på grener med hhv. blader og rakler, og lukket dermed inne eventuelle sugere som ville utvikle seg på grenene. Ytterligere innkapslinger ble satt på grener med blader i juli. Alle innkapslinger ble samlet inn i august, etter at de eventuelle sugerne ville ha utviklet seg til voksne individer. De innsamlede prøvene ble målt og opptalt i laboratoriet, etterfulgt av statistiske analyser for å finne trender og for å sammenligne dataene med 1995-funnene.

Noen forskjeller ble funnet i eggenes plassering på buskene, men funnene var ikke signifikante. Antall egg på raklene hadde en betydelig sammenheng med raklenes utviklingsstadiet og deres observerte tetthet. Bare én av sugerartene ble funnet i de nye prøvene av voksne individer, og deres gjennomsnittlige antall var betydelig lavere for de fleste innsamlingssteder i 2018. Jeg tolker forskjellen i resultatene som en effekt av endret fenologi fra 1995 eller at metodene er litt forskjellige. Temperaturøkningen vil trolig også være en del av forklaringen, da sommermånedene i 2018 var unormalt varme og solfylte.

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1. Introduction

Psyllids (Homoptera: Psylloidea) are insects feeding on the phloem of a small number of closely related host plants, often within a single genus, and their life cycle is tightly connected to air temperature and the development cycle of their host plant (McLean and Hodkinson 1980).

Rise in global temperature has affected many ecosystems across the world throughout the past few decades (IPCC 2007). The mean range shift of species has been calculated to 6.1 km per decade towards the poles (Parmesan and Yohe 2003). By comparing the mean temperature of the summer months (June-August) in Norway throughout recent years, many summers are found to be above normal temperature, with 2018 at 1,8 °C above normal, and 3-4 °C above normal in the eastern parts of Norway where we have the location of this study (Grinde 2018).

A thick, protective layer of snow protects the insects from potentially lethal winter temperatures; the temperature under the thick snow rarely drop below 0 °C (Hodkinson and Bird 1999). Higher temperatures can extend the summer season, which might play a big role in insect phenology and distribution, including an increased number of generations per season. As herbivore life-cycles are often synchronized with the phenology of their host plants, the changes often are restricted to and follow in synchrony with changes in host plant phenology (Bale et. al. 2002).

The downy willow *Salix lapponum* is prevalent in the mountain areas of northern palearctic. The different species of psyllids are relatively restricted to particular elevations in the mountains with some overlap of the species along an altitudinal gradient, using several different species of *Salix* spp. as host plants (Ossiannilsson 1992).

A study of the presence and abundance of *Cacopsylla* spp. on *Salix lapponum* were carried out during the summer of 1995 in an altitudinal gradient from Haugastøl to Finse in the alpine areas of southern Norway (Hill et. al. 1998). The study found three *Cacopsylla* species along the gradient with *C. propinqua* covering the entire altitudinal range, while *C. brunneipennis* was mainly found in the lower sites, and *C. palmeni* mainly in the higher sites. All species developed in varying degrees on both the leaves and catkins of the *Salix*.

With global climate change, the phenology of some insects and host-plants is assumed to change, and the distribution to have moved to higher latitudes and altitudes (Parmesan 2006). To see if I could see effects of the changes in the insect abundance and insect-host relationship in the previously studied area, I resampled the same sites in 2018 closely following the methodology of the Hill et. al. 1998 study, which was done in 1995. The environment in this area could presumably have become less suitable for the higher-dwelling species. The temperature, among other environmental cues, has implications on development in insects as well as in host plants of phytophagous insects (Gilbert 1980). Change in temperature might result in an offset in synchrony between insect and host plant, which can lead to further alterations in the insect population (Hill 1995).

Previous resampling studies have shown changes in relationships between individuals within a species as well as plant-herbivore interactions, and subsequent decreased resilience to disturbance in plant and insect species over as much as a decade (Burkle et. al. 2013). Some studies have shown phenological responses with climate warming generating mismatches among the partners of mutualistic relationships (Hegland et. al. 2009).

Through modelling studies, several species with different ecosystem functions are shown to be affected, with arctic-alpine communities appearing to be one of the most sensitive ecosystems to climate change effects. Inhabiting species in arctic-alpine areas could be gaining and losing suitable living conditions over time (Berry et. al. 2008). Changes in the organization of the herbivore community can occur as well, and new species not previously found can appear over time (Walther 2010). Many aspects of the changes and consequences are still unexamined and unknown, as knowledge of mutualistic plant-herbivore relationships is lacking, and there will be more research required in this field (Li et. al. 2011).

I will compare the 1995 data described in the Hill et. al. paper to current year findings. I resample the same area and the same 7 sites from Haugastøl to Finse and investigate whether there are changes in the distributions of psyllids on *Salix lapponum*, by comparing the results from the previous sampling. With the increase in air temperature leading up to 2018 compared with 1995, I predict to see the following changes in the distribution of the psyllid species:

Eggs on *Salix*

- I investigate if the location of egg laying and development in the *Salix* plants has changed, and predict that there will be a trend towards eggs increasingly being laid and developed on leaves rather than catkins, as the protection of the catkins will be less important with increased air temperature.
- When the catkin density increases, and the catkins and shrubs are further developed (catkin developmental stage, density and shrub height), making it more suitable for egg laying, I should see a significantly increased abundance of eggs.

Adult psyllids

- I predict to see a difference in adult psyllid species composition, with the species mainly found in lower altitude (*C. propinqua* and *C. brunneipennis*) increasingly inhabiting sites in the higher altitudes and subsequently declining in the lower altitudes. The most generalistic species (*C. propinqua*) is expected to expand its habitat and abundance.

2. Materials and methods

2.1 Study area and species

The methods used were based on the methods described in Hill et. al. 1998. I followed them as close as possible, and the reasons for differences are explained. Data collection were done at the same 7 sites described in Hill et. al. 1998 reaching from Haugastøl to Finse from June-August 2018. They are situated along a 27 km stretch of the historic Rallarvegen road and the Bergensbanen railway track (Figure 2.1).

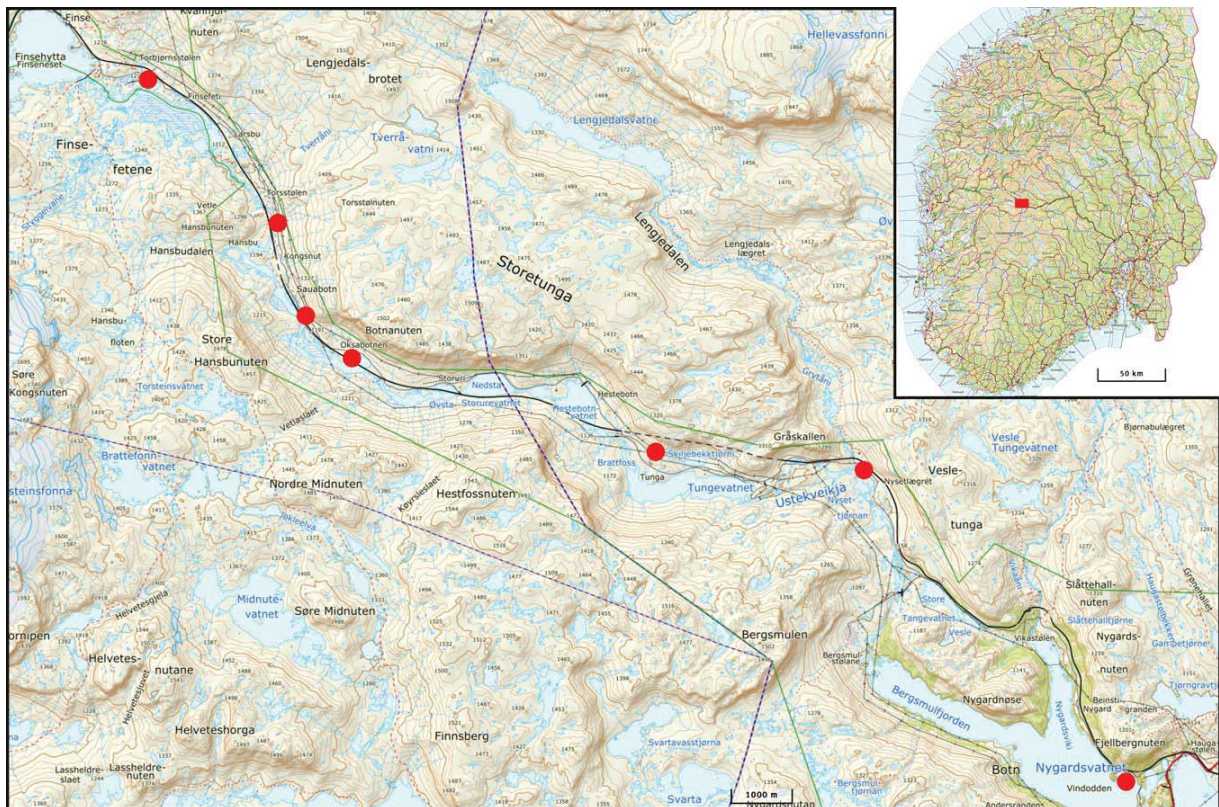


Figure 2.1: Map of study area with sites marked, and location in Norway. (Kartverket 2018)

All sites are located in a low alpine landscape between 998 and 1222 m a.s.l. (Figure 2.2) in the cold climate zone at 60° N. The mean summer temperature measured at Finse observation site is

8.9°C between the years 1900 to 2018, with the years 1995 and 2018 measured at 9.1°C and 10.9°C respectively (Norwegian Meteorological Institute 2018).

Vegetation at the sites is predominantly *Salix spp.*, with Haugastøl being the only site below the tree line. I registered the psyllids on *Salix lapponum*, which reproduces with hairy catkins and provide suitable egg laying conditions for psyllids on both the catkins and leaves.

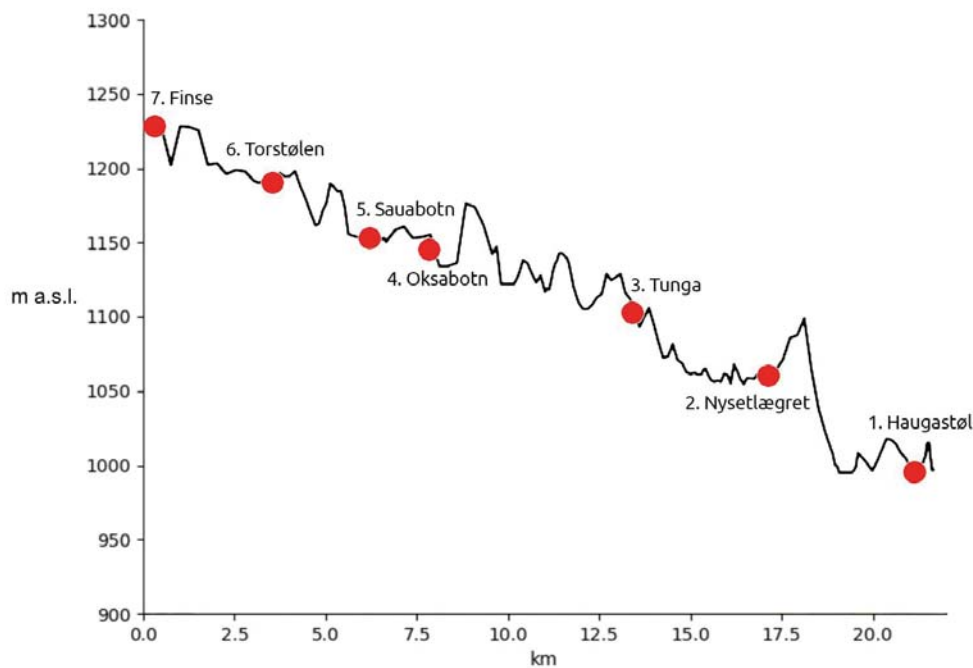


Figure 2.2: Elevation gradient across the transect from Haugastøl to Finse. (Kartverket 2018)

2.2 Data collection

I located 10 *Salix lapponum* individuals for each site on June 5th-6th 2018, within a relatively small area, and only individuals with minimum 10 branches with catkins on were chosen. The sampling in 1995 had 30 shrubs in site 1 and 7, which could result in less balanced data between sites. I chose to pick out the same number of shrubs for each site to provide uniformity in the results - a total of 70 shrubs - as opposed to 110 shrubs in the 1995 sampling.



Figure 2.3: Top left: *Salix lapponum* at site 6, Torstølen (June 6th 2018). Top right: *Salix lapponum* at site 5, Sauabotn (July 22 2018). Bottom left: Closeup of enclosure at site 2, Nysetlægret (June 6th 2018) Bottom right: Closeup of *Salix lapponum* at site 2, Nysetlægret (July 22 2018).

The height and diameter of the shrubs were measured in addition to the density of catkins by counting the number of catkins in five randomly placed 30 x 30 cm squares for each shrub. Two tan nylon stockings, about 20 cm long, were put on separate branch tips, one exclusively containing a branch of 2-4 catkins and one exclusively with leaves (Figure 2.3). The excess catkins or leaves were picked off the branches before the stocking enclosures were put on and fastened with cable ties onto the branches. From each individual *Salix* I picked and collected 10 female catkins and 40 leaf shoots.

The enclosures were applied after *Cacopsylla spp.* egg-laying was assumed to be done for all sites, to ensure that the insects were trapped inside. One additional enclosure with leaves were added to every individual shrub on July 22nd to account for any eggs laid later on the leaves. All branches with enclosures were cut and collected August 26th.

Total number of nymphs and eggs on catkins and leaves from each of the samples collected in June were counted in the lab. Adult psyllids were counted and identified from the enclosures collected in August. All samples were examined thoroughly in a magnifier, finding and counting all *Cacopsylla spp.* eggs and nymphs (Figure 2.4).

Ian Hodkinson, who identified the adult psyllid species in the lab work for Hill et. al. 1998, identified the psyllids. The nymphs and eggs are not easily identified to species level and was not further identified for either the 1995 or 2018 study.

The catkins were given a phenological score between 1 and 10 where 1 is pre-flowering and 10 is after seeding, by observing the catkins and measuring the ovaries (Table 2.2: Hill and Hodkinson 1995). The median stages from each site were calculated and used in statistical tests.

Table 2.1: Total number of shrubs sampled, number of catkins, leaf buds and enclosures (stocking bags) collected for each site.

Site	Shrubs sampled	Catkins and leaf buds collected June	Bags applied in June	Bags applied in July	Bags collected in August
1-7	10	10x10 catkins, 40x10 leaf buds	10 catkins, 10 leaves	10 leaves	30 bags

Table 2.2: Phenological score of catkin developmental stage (Hill and Hodkinson 1995)

Stage	Visual description	Ovary length
1	Ovary hidden beneath bract.	2.5-3 mm
2	Style visible above bract.	3-4 mm
3	Length of visible ovary equal to length of bract.	4-5 mm
4	Style turning brown.	5-6 mm
5	Fertilized ovaries starting to swell.	6-7 mm
6	Unfertilized ovaries starting to wither.	7-9 mm
7	Catkin fully elongated; ovary fully swollen.	> 9 mm
8	Ovaries that are most mature starting to dehisce.	-
9	All ovaries fully dehisced.	-
10	Catkin starting to wither; all seeds released.	-



Figure 2.4: Top left: Adult *Cacopsylla propinqua* from site 3, Tunga (~25x magnification). Top right: *Cacopsylla* spp. nymph from site 3, Tunga (~25x magnification). Bottom left: *Cacopsylla* spp. eggs under *Salix lapponum* leaf from site 1, Haugastøl (~25x magnification). Bottom right: *Cacopsylla* spp. nymphs on *Salix lapponum* catkin from site 1, Haugastøl (~10x magnification).

2.3 Statistical analysis

Data processing was conducted in Excel for Mac (version 16.22). R software (version 3.5.1) was used for statistical analysis, and figures were made in RStudio (version 1.1.463) and Excel. Tables were made in Excel. With the R software I also calculated means and standard errors where used.

The statistical analyses were done as closely as possible with the approach of the Hill et. al. 1998 study to get comparable results. The data collected for the 1998 study were compared with 2018 data, I did not have the complete data sets from the study, but used approximate measures made from the figures used in the Hill et. al. 1998.

Catkin stage differences between sites were tested with a paired T-test, as well as the testing of the difference in proportions of psyllid eggs on catkins over leaves and regression analyses to compare the psyllid egg abundance to shrub developmental indicators.

Three shrub developmental indicators: catkin density, catkin stage and shrub height were tested together with the number of eggs on the catkins with an adjusted multiple regression test.

The 1995 and 2018 data sets for collected adult psyllids were compared with a Wilcoxon signed rank test.

A one-way ANOVA test between all sites along the gradient were done to compare adult psyllid abundance of each site, and the test of adult psyllid abundance effect with height above sea level were done with a Pearson's product-moment correlation test.

3. Results

A total of 4274 eggs and 7876 nymphs were found in the samples of catkins and leaf buds collected in June (a total of 700 catkins and 2800 leaf buds) along the gradient distributed between 7 sites from Finse to Haugastøl. A total of 507 adult psyllids were collected in August on *Salix* caged in June and July. All adult individuals collected in 2018 were determined to be of the species *Cacopsylla propinqua*.

3.1 Psyllid eggs on *Salix*

Psyllid eggs were found on leaves and catkins collected in June at site 1, 2, 3 and 5. A higher proportion of eggs were now found at sites 3 and 5, and sites 1 and 2 had the lowest proportions. The proportions of eggs on catkins from 1995 compared with those found in 2018 was not significantly similar (Figure 3.1: $t = 0.38$, $df = 6$, $p = 0.71$). Site 4, 6 and 7 did not have eggs in the 2018 sampling, and site 4 did not have any eggs in samples collected in 1995.

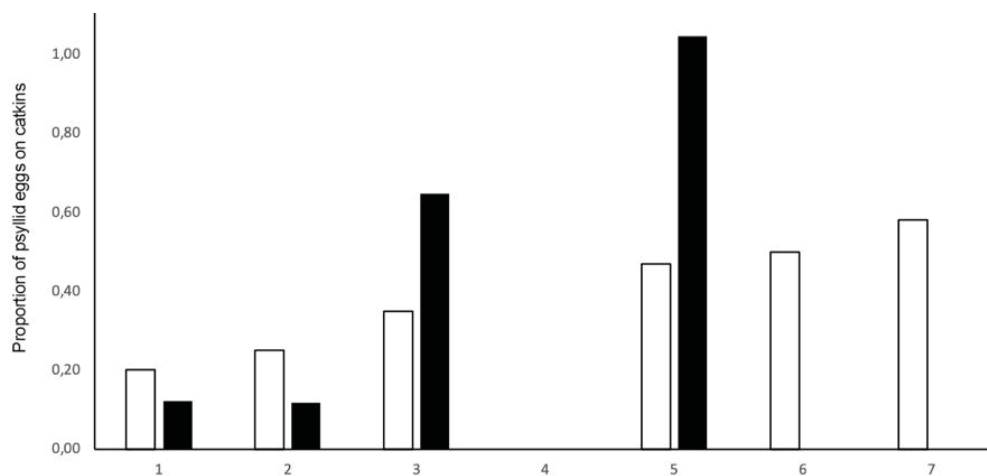


Figure. 3.1: Proportion of eggs found on catkins compared with eggs on leaves in site 1-7. Open bars: data from 1995. Black bars: data from 2018.

The number of eggs per catkin had a significant relationship with the phenological stage of the catkin. (Linear regression, $N = 945$, $t = 4.0$, $p < 0.001$). The psyllid egg abundance also had a significant relationship with the density of catkins on the shrubs (Regression: $N = 4274$ (2018), $t = -4.7$, $p < 0.001$). The density is shown in a box plot in figure 3.3.

The development stages of catkins collected in early June had a significant correlation with site height above sea-level (Figure 3.2: $t = -132.06$, $df = 69$, $p < 0.001$).

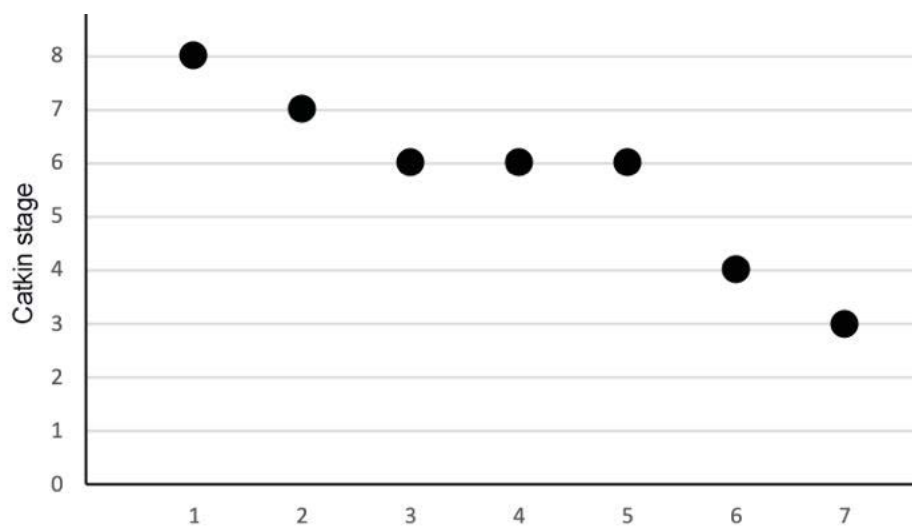


Figure. 3.2: The median phenological stage of catkins per site 1-7 when collected in early June 2018.

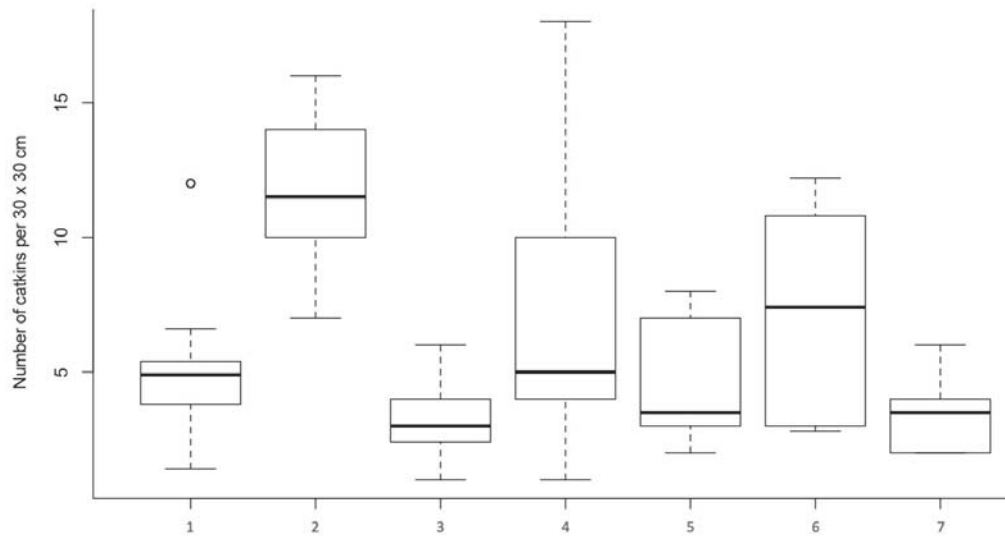


Figure. 3.3: Catkin density calculated as the mean of 5 measurements inside 30 x 30 cm randomly picked squares on each of the 10 shrubs per site on the sampled Salix individuals in the 7 sites. 70 shrubs were measured with a total of 350 measurements. The lines in the boxes mark the median, the boxes illustrate the 1st to 3rd quartile of means and the dotted lines show the maximum and minimum mean for each site, with one possible outlier mean at site 1.

Egg abundance had a significant relationship with the heights of the shrubs they were sampled from ($N = 70$, $t = -4.7$, $p = 0.05$, mean shrub height per site 1-7 is illustrated in figure 3.4). Catkin density, catkin stage and shrub height tested together with multiple regression had a significant relationship with the number of eggs on the catkins (Table 3.1.1: $N = 70$, Adjusted multiple R -squared = 0.07, $p < 0.001$).

Table 3.1.1: Multiple regression of eggs per catkin in relation to catkin density and stage, and shrub height. Significant results: < 0,001***, < 0,01**, < 0,1*.

Variable	Estimate	SE	t-value	p-value
Intercept	-0,57	0,44	-1,3	0,2
Catkin density	-0,15	0,33	-4,7	< 0,001***
Catkin stage	0,37	0,09	4,0	< 0,001***
Shrub height	0,75	0,38	2,0	0,05*

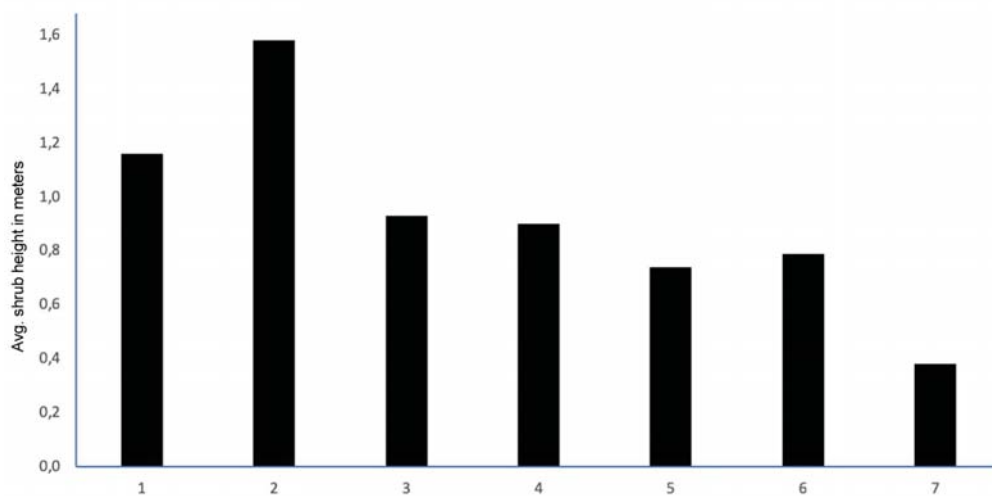


Figure. 3.4: Mean height of Salix lapponum shrubs per site 1-7 in meters.

3.2 Adult psyllids

No adult individuals were collected from the highest located site, Finse. The difference in abundance was statistically significant between the sites in 2018 (Figure 3.5: ANOVA, $F = 17.71$, $p < 0,001$), with a tendency towards higher abundance in the mid-to-lower sites, but no significant relationship between abundance and height above sea level ($t = -0.29$, $df = 68$, $p = 0.77$). The mean abundance of adult psyllids were higher for every site except site 4, Oksabotn, in 1995 when comparing to the mean abundance in the 2018 data, the findings from the two years were not significantly similar when testing the means against each other ($p = 0,81$).

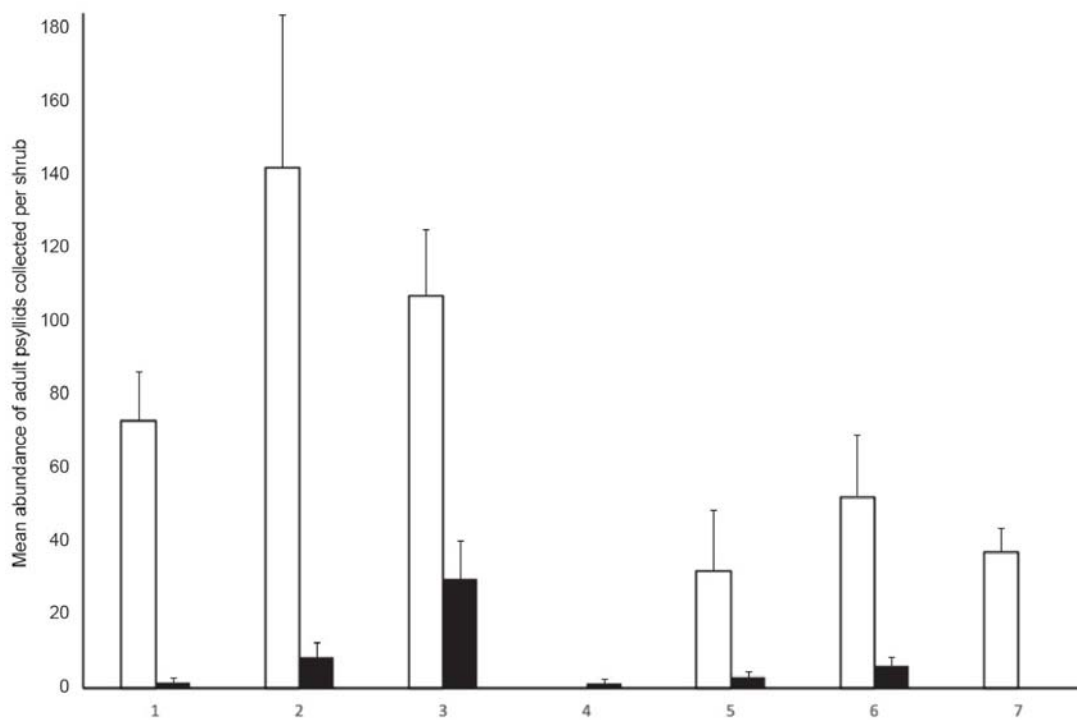


Figure. 3.5: Mean abundance of adult *C. propinqua* collected in August on *Salix* in enclosures, as distributed on site 1-7, number per shrub. Open bars: 1995 sampling. Solid bars: 2018 sampling. 2018 data from 70 individual shrubs, 1995 data from 110 individual shrubs.

4. Discussion

Similar to the proposed hypothesis, I found a difference from 1995 data in psyllid eggs increasingly laid on leaves at the middle sites 3 and 5, Tunga and Sauabotn. At the other sites there were little difference. The abundance of eggs on catkins increases significantly when the shrubs are bigger, catkin stage development has developed further and catkin density is higher, this is apparent both when the variables are tested together and when one at the time. Catkin stage had a significant relationship with height above sea level.

For adult psyllids, I found a difference from 1995 data in species composition throughout the altitudinal transect, as predicted in one of the hypotheses. Only one of the three species from the previous study, Hill et. al. 1998, were collected in 2018. The catkin developmental stages also had a significant correlation with height above sea level.

4.1 Psyllid eggs on *Salix*

The relatively high summer temperature in 2018 might be an advantage for the species that is more suited for utilizing leaves for egg development, as catkins are particularly suitable for egg development in lower temperatures. The insulating effects of the enclosures on the branches or of the catkins themselves might make a too hot environment for the eggs to successfully hatch or develop. This can mean the other species, which are more dependent on developing on catkins, will be worse off. At the middle sites 3 and 5, Tunga and Sauabotn, proportion of egg-laying on leaves were even higher in 2018 than in 1995. Fewer hatchings and reduced viability have been shown in eggs of the mosquito *Anopheles gambiae* in too high or too low temperatures (Impoinvil et. al. 2007). There was a significant relationship between catkin developmental stage and height above sea level, so the catkins in the higher sites are later developed and smaller than in the lower sites, resulting in less suitable conditions for nymph development in the higher sites (Hill and Hodkinson 1995).

In lower sites more of the plant tissue, i.e. leaves, can be used for the hatching and development, which *C. propinqua* utilizes especially, with 80 % of sampled individuals in the 1995 sampling developing on leaves, compared with < 10 % for the other species (Hodkinson and Bird 1999, Hill et. al. 1998).

4.2 Adult psyllids

Similar to the findings in 1995, the adult psyllid abundance across the different sites varied significantly, with a slight inclination to shift of abundance when comparing site 2 and 3, Nysetlægret and Tunga. Although there were variations between sites, there was no distinctive change in the abundance of psyllids in relation to height above sea level. The one adult psyllid species identified, *Cacopsylla propinqua*, is associated with low to high alpine areas across western Palearctic, from central Europe and the mountains of the Caucasus in the south, with the species inhabiting lower altitudes when going north towards the Arctic (Conci et al. 1993).

An explanation to *C. propinqua* being the only particular adult species collected can be found in the 1995 data. The species was found to be more generalistic compared to the other species in terms of distribution throughout the gradient as well as nymph development, and it is more suited to lay eggs on leaves rather than just catkins compared with similar psyllid species (Hill et. al. 1998). *C. propinqua* was also found to be the most locally abundant and with the longest range, and these species with broader niches are associated with larger range and abundance (Brown 1984).

From one year to another short-term changes in weather and living conditions may account for fluctuations in abundance that are found doing resamplings. The differences between years can be more easily explained when closely following the same sample methods every year (Alarcón et. al. 2008). As we only have one year of resampling, both the deviation in date of putting on the containers on the shrubs and year-to-year seasonal variation can account for some of the difference between samplings.

Contrary to my assumption, attaching the enclosures may have been done too early in the season for the higher located sites, as the enclosures were attached . This was accounted for with additional enclosures in July, which still gave small numbers of adults overall compared with the 1995 study, and no adults were developed in the samples in the highest site, Finse. The enclosures were attached mainly between June 5th and 6th, while in 1995, they were not attached before June 18th. However, the July attachment were done with only 1-3 days difference from the original study. A more probable explanation might be that there was an effect on the egg development within the enclosures from the drought or heat in Eastern Norway in the summer of 2018 (Grinde et. al. 2018).

I collected only female catkins, which are shown in *Salix arctica* to effectively absorb sunlight, resulting in higher temperature than male catkins (Kevan 1990). If this can be transferable to *S. lapponum*, this may be the reason nymph development might be doing better in low altitudes where the catkins are further developed.

4.3 Conclusion

Higher air temperature may be one of the decisive explanations for our observed changes from the 1995 findings, and the psyllid population may be utilizing leaves even more when facing rising temperatures. With higher temperatures and CO₂ levels, the shrubs could expand into even larger areas, develop earlier and with more catkins which are suitable egg-laying places. As long as the temperature is within the range of egg-hatching temperature, some of the changes discovered points to possibilities for the psyllids to increase in abundance and expand further. Especially the generalist *C. propinqua* could be leading the growth, based on this resample.

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Appendix

Table A.1: The sites from east to west across a low to high altitude gradient.

Transect	Transect name	Latitude	Longitude
1	Vindodden, Haugastøl	60° 30.6393491' N	7° 50.3102691' E
2	Nysetlægret	60° 33.0717249' N	7° 45.0405958' E
3	Tunga	60° 32.9319659' N	7° 41.6559140' E
4	Oksabotn	60° 33.3026491' N	7° 36.5976975' E
5	Sauabotn	60° 33.9931350' N	7° 34.7457163' E
6	Torstølen	60° 34.7418331' N	7° 34.2511010' E
7	Finse	60° 35.6327033' N	7° 31.3781820' E



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