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A three-year population analysis of the red listed musk orchid (*Herminium monorchis*) in South-East Norway

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Master of Science in Ecology

PREFACE

Firstly, I would like to give my thanks and gratitude to my supervisors Kari Klanderud (MINA, NMBU) and Marianne Evju (NINA) for their great mentoring both during my field work and during the writing and statistical analysis. Thanks to MINA at NMBU and NINA for letting me lend their field equipment. I also want to give thanks to the Fylkesmannen i Østfold for giving me financial support for my field work as well as good communication. I would also give thanks to Liv Ingrid Kravdal for letting me use her field diagrams.

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SUMMARY

The combination of abandonment and intensification of agriculture has changed the agricultural landscape, especially the semi-natural nature types. This has led to a loss and endangerment of species linked to these nature types. The musk orchid is one of these species. In Norway, the species is now only found in Hvaler municipality, South-East Norway, and is listed as critical endangered (CR) in the Norwegian red list.

My master's thesis is a part of an ongoing project that started in 2014 by a former master student, where I collected data in 2016 and analyzed the datasets from 2014 to 2016. The purpose of this thesis is to get a better understanding of how the last populations of musk orchid in Norway are performing, which the management authorities can use in further management. To answer this I had three main questions; i.e. (1) how the environmental factors are differing between the three locations and through the three years, (2) how the musk orchid population performance at the three locations differed over the three years, and (3) how grazing affects the two sub-populations, at one of the locations, three years after grazing ended.

My results suggest that both the abiotic and biotic environmental factors are impacting the three locations differently, and the performance of the musk orchid are correlating this. The smallest individuals and lowest fertile proportion of musk orchid are found at the driest location, which also had the lowest surrounding vegetation. At the wettest location, the trend is the opposite. It also seems like grazing influences the musk orchid even three years after the area has been grazed the last. The musk orchid in the grazed sub-population is larger and have a higher proportion of fertile plants.

In further management of the musk orchid at Hvaler it is crucial to continue the monitoring, to detect the whole population ant its dynamics. Another important management mean is to either hay or have grazing that will open and keep the surrounding vegetation down. But before any reintroduction of grazing at the locations which are not currently grazed, it is important to find a livestock density, size or race that will not hurt the musk orchid populations, as well as excluding grazing when the musk orchid is in the fertile stage.

Key words *Musk orchid, Herminium monorchis, nature management, population analysis, red listed species*

SAMANDRAG

Kombinasjon av opphøyr og intensivering av jordbruksland har endra jordbrukslandskapet, og spesielt dei semi-naturlege naturtypane. Denne endringa har resultert i eit biomangfaldtap og trugar artar som er assosiert til desse naturtypane. Honningblomen er ein av desse artane. I Noreg er arten no berre funnen i Hvaler kommune, Søraust Noreg, og er lista som kritisk truga (CR) på den norske raudlista for artar.

Eg fortsette eit prosjekt som byrja i 2014 av ein tidlegare masterstudent, der eg samla data i 2016 og analyserte datasetta frå 2014 til 2016. Føremålet med oppgåva mi er å få ei betre forståing over korleis dei siste honningblom-populasjonane presterer, slik forvaltinga kan bruke denne informasjonen til vidare forvalting. Dette vart gjort ved å spørje tre hovudspørsmål; (1) korleis miljø faktorane varierer mellom dei tre ulike lokalitetane og mellom dei tre åra, (2) korleis planta presterte i dei tre ulike lokalitetane mellom dei tre åra, og (3) korleis beiting påverkar, sjølv etter opphøyr av beiting i to sub-populasjonar i ein av lokalitetane.

Resultata mine tilseier at dei tre ulike lokalitetane har forskjellig påverknad frå både dei biotiske og abiotiske miljøforholda eg målte, og at honningblom prestasjonen korrelerte med disse. Den tørraste lokaliteten hadde den kortaste omliggjande vegetasjonen, her var honningblomen minst og hadde den lågaste fertile andelen av dei tre lokalitatetane. I den våtaste lokaliteten var trenden motsett. Det ser òg ut til at beiting har ein effekt på honningblom, sjølv tre år etter at beitinga opphøyrde. Honningblom i den beita sub-populasjonen hadde større planter samt høgare proporsjon av fertile planter samanlikna med den andre sub-populasjonen.

For vidare forvalting av honningblom på Hvaler er det særs viktig å fortsette overvåkinga, for å oppdage heile populasjonen og dynamikken i den. Det er òg viktig å anten drive med slått eller beiting som vil kunne opne opp og halde vegetasjonen nede. Før ein eventuelt gjeninnfører beiting i dei lokalitetane der det ikkje er beiting nå, er det viktig å finne ein beitedyrs- tetthet, størrelse og type som ikkje vil skade honningblom populasjonane. Samt halde beitedyr vekke når honningblom er i sitt fertile stadie.

Nøkkelord *Honningblom, Herminium monorchis, naturforvaltning, populasjons analyse, raudlista artar*

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INTRODUCTION

In the last 50 years, there has been a combination of abandonment and intensification of agricultural landscapes in Europe, where the semi-natural habitats are disappearing (MacDonald et al. 2000). Abandoned semi-natural habitats will lead to overgrowing. This may lead to a succession into forested areas or shrub-lands (Dicks et al. 2014; MacDonald et al. 2000). According to a study by Staaland et al. (1998a), the time for regrowth after abandonment of the farmland might depend on how far it is to the closest forest edge and environmental factors such as how wet the habitat was before it was abandoned. Staaland et al. (1998a) discuss therefore that an abandoned open grass area might become overgrown as fast as after 20 to 30 years. Intensification also influences the biodiversity in semi-natural areas as the management of the area shifts into other types of agricultural managed areas (Dicks et al. 2014). This may lead to soil erosion and negative effects on the nearby environment. These changes in the agriculture has led the loss of semi-natural habitats and the mosaic landscape to shrub lands, forests and the development of houses, roads or other types of infrastructure (Dicks et al. 2014; MacDonald et al. 2000).

Semi-natural areas often have high biodiversity and are therefore important in the agricultural landscape (Hietala-Koivu et al. 2004). An example is hay meadows, as many species are attached to them, and some of the species are specialized to grow and live here (Direktoratet for naturforvaltning 2009). A group of species that are threatened by the land use change are orchids, especially in wet meadows, as they often are restricted due to their niche specificity or barriers that reduces their dispersal potential (Swarts & Dixon 2009; Wotavova et al. 2004). Therefore, orchids are one of the first species that will disappear from disturbed systems (Swarts & Dixon 2009). Disturbances such as fertilization are favoring faster-growing nitrophilous species, which leads to exclusion of slow-growing species (Honsova et al. 2007; Maskell et al. 2010). Overgrowing from either the nitrophilous species or a change in the haying/grazing management of the meadow, will limit the light availability and therefore pose a major threat to slow growing species.

The abandonment and intensification trends are also occurring in Norway (Losvik 2003; Olsson et al. 2000; Staaland et al. 1998a; Staaland et al. 1998b). An example of an orchid that has been affected by the land use change is the musk orchid (*Herminium monorchis*). In Norway this species could be found at many locations in South-East Norway prior to 1900, however now it is only observed in a few locations in Hvaler municipality, South-East Norway (Miljødirektoratet 2010). Important negative factors may be fertilization, change in haying and grazing practices, and drainage of mires, as the musk orchid is a slow growing and small plant (Ekstam & Forshed 1992; Miljødirektoratet 2010; Økland & Økland 1996). Today it is a critical endangered (CR) species in Norway, however in Europe the red list status is data deficient (DD) (Bilz et al. 2011; Miljødirektoratet 2010). As a result of the decline it got a management plan in 2010, and in 2011 it became a priority species (Norwegian: prioritert art) in Norway (Miljødirektoratet 2010).

In management of orchids it is important to have knowledge about their ecology. Important knowledge includes; how the species respond to variation in spoil properties or vegetation coverage, which fungi species it forms mycorrhiza with, or important pollinators (Miljødirektoratet 2010; Swarts & Dixon 2009). An essential tool in the management is mapping and monitoring during and after the management occurs, to verify if the management had any effect (Miljødirektoratet 2010). If there is insufficient data, it will be hard to see if the environment is affecting the population dynamics of the plants.

Many perennial plants, including orchids, have an underground state where there is no aboveground biomass (Shefferson et al. 2014). Some orchids even get carbon from mycorrhizal fungi during this dormant stage (Bidartondo et al. 2004) As the cost of reproduction and production of leaves is high, it forces many perennial plants to stay in a vegetative state the following season, when their leaves have been too small to efficiently collect energy. When the size of the leaves are big enough they can break out of the vegetative state and reproduce (Primack & Stacy 1998). Wells et al. (1998) found this trend for the musk orchid as well, and additionally found that temperature and precipitation influences the size of the leaves. Wells et al. (1998) also hypnotizes that the drought increases the chance for smaller leaves that don’t produce enough carbohydrates for a successful flowering for both the current and the following season. The musk orchid has both an aboveground and an underground vegetative state. In the underground state there is only the root tuber left, and during aboveground vegetative state it may have different numbers of leaves (Wells et al. 1998). As observational studies often only count the aboveground individuals, it may not give a good insight in how the population size really is. Therefore, it is crucial to study the same individuals for several growing seasons. And by checking the same individuals for several years you can detect the plants in their underground state, and get a greater overview over the population. This method is beneficial because it will get an impression if the population is in fact increasing or decreasing. Another aspect to look at the ratio between vegetative and fertile individuals of the population, and to investigate what factors might affect flowering (Oostermeijer et al. 1994; Primack & Stacy 1998).

The nitrophilous species and shrubs can be hold at a height that the musk orchid can grow in if the area is grazed or cut regularly (Ekstam & Forshed 1992; Janeckova et al. 2006). Ekstam and Forshed (1992) states that due to these factors the musk orchid will be one of the first plants that decreases in numbers short time after any change in management. The musk orchid is connected with long-time managed pastures, and they thrives better here than in hay meadows (Ekstam & Forshed 1992).

The Miljødirektoratet (2010) management plan has discussed if there should be grazing at the musk orchid locations, Skipstadsand, Skjellvik and Teneskjær, in Hvaler. Cattle has previously grazed at all the musk orchid locations in Hvaler, but now only at two of the three locations. In Skipstadsand they cut the meadow during the summer so it won't overgrow. In Skjellvik and Teneskjær there are grazing cattle. The area around the musk orchid population in Skjellvik has been fenced in. This was done to reduce the damage made by grazing cattle on the soil, as the soil here is being destroyed by the type of cattle that are currently grazing the area. At first only a part of the musk orchid population was fenced in, this was done to see if it had any positive effect to exclude grazing at the musk orchid population in Skjellvik.

The objectives for this master thesis is to increase our knowledge of population variation in three of the remaining populations of musk orchid. To do this, I use data from 40 permanent plots marked in 2014 and analyzed from 2014 to 2016. The dataset includes 3-year data on the number of aboveground individuals, their size and number of flowers, and environmental factors for each plot. Specifically, I ask;

1. **How do the environmental factors vary?** Is there any difference between the locations and between years for the environmental variables? To answer this question, I measured an abiotic factor; soil moisture content, as well as two biotic factors; the height and the percentage of vegetation cover surrounding the musk orchid.

2. **How does the population performance of musk orchid vary on the different locations?** Has the performance of the musk orchid differed over the three-year period? Is there any difference between the three locations? To answer these questions, I

measured the density of musk orchid per plot, the size of the individual plants, if the plants are fertile or not and number of flowers.

3. **Does grazing of livestock matter for the performance of the musk orchid?** How will the musk orchid performance of sub-populations the inside- and outside of the fence at Skjellvik differ, and does the environmental factors differ where the two sub-populations grow? To answer this question, I examined both the plant performance variables as well as the environmental factors between the two sub-populations.

This information is aimed to be used to further management and conservation of the musk orchid.

MATERIAL AND METHODS

STUDY SPECIES

The musk orchid *(Herminium monorchis),* honningblom in Norwegian, is an orchid in the *Herminium* genus. This genus consists of 30 species which is found throughout Eurasia, however the musk orchid is the only one which grow in Europe. The musk orchid can be found from western Europe to China and Japan in the East (Willis & Shaw 1973).

The musk orchid is a perennial plant, with dormant stages underground (Wells et al. 1998). It has normally two leaves close to the ground, and one slim flower stem with several flowers (Fig 1). The flowers are pale yellow/green with a honey smell and nectar production (De Hert et al. 2013; Lid & Lid 2005). The keel of the flower is egg shaped with two side-flaps (Lid & Lid 2005). It spread both clonally and with seeds. When it spreads clonally, it produces stolons in the top of the root tuber that will produce a new root tuber (Lid & Lid 2005; Miljødirektoratet 2010).

The musk orchid is calciphilous, moderately salt, moisture and pH tolerant. It can be found in wet hay meadows, pastures, calcareous mires and on beaches (Økland & Økland 1996).

Figure 1 Picture of the musk orchid (*Herminium monorchis*) Photo by Fosse (2012).

STUDY AREA

There are three locations which I study the musk orchid, all of them are located on Asmaløy in Hvaler municipality in South-East Norway. In 2016, the warmest month was June, where average temperature was 15 C° (Meteorologisk institutt 2017b). The coldest month was in February, where the average temperature was 0,9 C° (Meteorologisk institutt 2017b). Asmaløy experience most of the precipitation during the fall, with the average of 100 millimeters in October, the least amount of precipitation occurs in February with only 40 millimeters (Eklima Undated). The temperature data are from Strømtangen fyr weather station in the neighbouring municipality Fredrikstad (Meteorologisk institutt 2017b). The precipitation data are from the official weather station on the neighboring island Kirkeøy (station no. 1050) for the normal period of 1961 – 1990 (Eklima Undated).

Figure 2 Red star is where Asmaløy island is located in Norway. Map over the three locations at Asmaløy, orange star is Skipstadstrand, yellow star is Teneskjær and blue star is Skjellvik. Map over Norway is from Google (2017), and map over Asmaløy from Miljødirektoratet (2017)

SKIPSTADSAND

Skipstadsand is located North-East on the Asmaløy island (Fig 2 and 3), and is categorized as a semi-natural salt meadow (Miljødirektoratet 2016c). In 2010 Skipstadsand became a Nature reserve, and is now managed by the County governor of Østfold (Fylkesmannen i Østfold

Figure 3 Picture over Skipstadsand in July 2016. Photo: Silje Skjelnes Vågen.

2013). Prior to 1965, the area was grazed by cattle, and since 2003 the inner parts of the meadow have been cut yearly to prevent the meadow to overgrow (Fylkesmannen i Østfold 2013). The musk orchid population size has varied a lot over the years, with the highest number recorded in 1996, with no individuals found in 2003 and 2004 (Fylkesmannen i Østfold 2013). The meadow is actively used by people, as it is near a cottage area (personal observation).

TENESKJÆR

Teneskjær is located in the South-West of the Asmaløy island (Fig 2 and 4), and is included in the Ytre Hvaler National Park. Teneskjær is managed by the National Park Board. The area is the driest location, with a shallow layer of shell sand, and it is categorized as a semi-natural salt meadow (Miljødirektoratet 2016b). Since 2009, cattle have been grazing this location during the summer months (Miljødirektoratet 2010).

Figure 4 Picture of Teneskjær in July 2016. Photo: Silje Skjelnes Vågen.

SKJELLVIK

Skjellvik is located in the South-West of the Asmaløy island (Fig 2 and 5), which is also a part of Ytre Hvaler national park. The area is near a pond and has a small brook running through one part of the area, and is categorized as a calcareous bog (Miljødirektoratet 2016a). In this location cattle have also been grazing during the summer months, however the management authorities fenced in the musk orchid population to protect it from the livestock. In 2009 only a part of the musk orchid population in Skjellvik was fenced, but in 2014 the whole Skjellvik population was fenced in, due to damage of the last part of the musk orchid population. The part of the meadow that was fenced in the last still show signs of tramping from the cattle (personal observation). Hence, this location is divided into two sub-populations, one ungrazed

Figure 5 Picture of Skjellvik in July 2016. Left side of picture is where the “outside of the fence” sub-population is located, and the right side is where “inside of the fence” sub-population is located. Photo: Silje Skjelnes Vågen.

and one grazed until 2014. This is done to see if the musk orchid bears any after-effects of the grazing. The area is cut yearly to prevent it to overgrow.

DATA COLLECTION AND DATA PROCESSING

The data was collected in the beginning of July for a three-year period (2014 to 2016). The first two years of the measurements were conducted by Kravdal (2015). In 2014, 40 permanent plots were established for monitoring of musk orchid (Kravdal 2015), 10 in Teneskjær and Skipstadsand and 10 in each of the sub-locations at Skjellvik. To locate the plots, the area was surveyed for musk orchid. Marking sticks were then placed at each individual/groups of individuals, and ten random marking sticks were selected to be the plots for further survey. Some of the plots were difficult to find again in the following field work seasons. In 2015, only 36 plots out of 40 was found and registered (4 missing from Skjellvik), and in 2016, 39 plots were found and registered (missing 1 in Skjellvik, in the "outside of the fence" sub-population).

For the environmental factor analysis, two biotic factors were included; percentage of vegetation cover and vegetation height, as well as an abiotic factor, soil moisture content. The percentage cover of woody plants, mosses, herbs, gramnoids, in addition to all vegetation combined were estimated for each plot in 2014 and 2016. Vegetation height was measured with a folding ruler in each of the outer corner of the inner four sub-plots (Fig 6) in 2014 and 2016. This resulted in four measurements per plot per year that were calculated into an average for each plot, both years. In 2014 and 2016, the soil moisture was measured with the soil moisture sensor SM300, in July. The measurements were done in the outer corner of the inner four sub-plots (Fig 6), this lead to four measurements per plot that were calculated to an average for each plot, each year. Due to malfunction in the soil moisture content instrument in 2015 both the abiotic and biotic environmental factors were not measured that year.

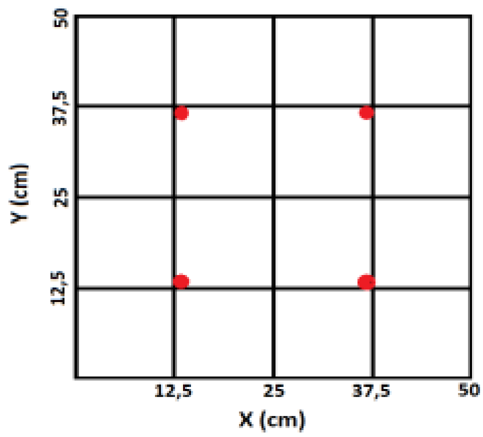

Figure 6 Illustration of a sampling plot. Each of the sampling plots are in total 50 x 50 cm, with 16 sub-plots that are 12,5 x 12,5 cm. The red dots are where the environmental factors were measured. Figure taken from Kravdal 2015.

To delimit the area for recording musk orchid individuals, as well as getting coordinates that could be used to track the same plant individual for several years, each of the sampling plots were divided into a 16-piece sub-plot net (Fig 6). The measurement of the musk orchid started at the lower left corner, and expanding to the next surrounding sub-plots if there was fewer than 20 individuals in the inner sub-plot. This continued until there were over 20 individuals in the accumulated sub-plots, and all the observed individuals in these sub-plots were counted and measured. For each individual I measured the length of the longest leaf and the width of the widest leaf in millimeters, as well as counting number of leaves. If the plant had a fertile stem I measured the height of the plant and the fertile part itself and counted the number of flowers. The measurements were done with a folding ruler.

To identify, the individuals were marked with numbered plastic toothpicks in 2015 and 2016 (Fig 7), in addition to written down coordinates on the field form for all years (Kravdal et al. 2016). However, the musk orchid often grow crowded and the aboveground part of the plant will not come up the same place from the tuber each year. This can make it difficult to know if it is the same individual you are counting for each year. For this reason, I did not use the information on identity in my statistical analyses.

Figure 7 How the musk orchid individuals were marked in 2015 and 2016 with numbered toothpicks. Photo: Silje Skjelnes Vågen.

STATISTICAL ANALYSIS

To explore the variations in both the abiotic and biotic environmental factors between locations and years, I conducted to types of tests. Firstly, I examined if there was any difference between 2014 and 2016 for the environmental factors, and how this potentially is correlating with the musk orchid performance. Each of the environmental factors were tested in separate tests, and was done separately for each of the three locations. I used linear mixed-effect models with plot as the random factor, to account for the repeated sampling of the same plots. The fixed factor for the model was year. Secondly, I used the dataset from 2016 to investigate differences between localities, for each of the environmental factors separately. I used a linear regression, followed by a post hoc Tukey test to differentiate between the three locations.

To investigate the variance in population performance over the three-year period, I analyzed the density of plants per plot, size of the plant, number of flowers per plant and if flowering occurred. Each of these variables were tested separately for each of the three locations. To calculate the size of plants I multiplied the width of the widest leaf, length of the longest leaf and the number of leaves, for each individual registered. The dataset of the size of the plants was so skewed that it was log10 transformed to compensate. For the analyses of the flower occurrence I specified the model to be binomial, and for the analyses on number of flowers I specified the model to be Poisson. The plant variables were also tested in mixed-effect models with plot as a random factor, and year as fixed factor. This was followed by post-hoc Tukey tests to see if there was any difference between the three years.

To examine if the fencing in Skjellvik has affected the environmental factors and plant performance variables I used mixed models in the same way as above. But instead of testing between the years, I looked at the difference between the two sub-populations, with the data from the three years combined.

All the statistical analyses and preparation of figures were carried out in the statistical program RStudio ver. 1.0.136. I used "lme4" for the generalized linear mixed-effects models and "nlme" for the linear mixed-effects models which I did not specify this. For the post-hoc Tukey tests I used the "multicomp" library. For all the models, I looked at "Q-Q plot" and "Residuals vs Fitted" to look for any potential outliers and verifying the assumptions for the models. All the bar plot figures were made with the sciplot library, I also used the plyr library to change the factor names for the grazing analyses figures.

RESULTS

ENVIRONMENTAL FACTORS AT THE MUSK ORCHID LOCATIONS

Teneskjær was the only location that had a difference in vegetation cover between 2014 and 2016, where the percentage of vegetation cover was declining (table 1 and 2). There was no significant difference in vegetation cover between the locations in 2016 (table 1, 3 and see appendix II).

Skipstadsand was the only location that had a difference in vegetation height between 2014 and 2016, where the height declined (table 1 and 2). In 2016, the vegetation was lower in Teneskjær as compared to Skjellvik and Skipstadsand, nevertheless there was no signifcant difference in height between Skjellvik and Skipstadsand (table 1, 3 and see appendix II).

All the locations had higher soil moisture content in 2016 as compared to 2014 (table 1 and 2). In 2016, Skjellvik was wetter than Teneskjær and Skipstadsand (table 1, 3 and see appendix II). However, there was no significant difference between Teneskjær and Skipstadsand in 2016.

Table 1: Mean and standard deviation (SD) of the measured environmental factors in 2014 and 2016.

Location	**Year**	**Vegetation cover (%)**		**Vegetation height (cm)**		**Soil moisture content (%)**	
		Mean	SD	Mean	SD	Mean	SD
Teneskjær	2014	92.20	5.86	3.60	0.97	15.78	10.35
	2016	78.00	19.17	2.93	1.11	34.03	15,36
Skipstadsand	2014	90.50	14.23	10.90	2.88	30.61	7.16
	2016	89.00	12.64	7.59	0.97	41.46	5.53
Skjellvik	2014	84.00	8.52	11.20	4.89	53.47	4.88
	2016	90.26	10.86	10.63	5.11	66.31	5.37

Table 2: Estimates and standard errors (SE) of the vegetation height, vegetation cover and soil moisture content between 2016 and 2014. The values are from mixed models that for each of the locations separately. 2014 is the reference year. Stars (*) indicates p-values, *** p<0.001; ** p<0.01; * p<0.05; . p<0.10. For pair-wise differences, see Appendix I.

Locations	**Year**	**Vegetation cover (%)**		**Vegetation height (cm)**		**Soil moisture content (%)**	
		Estimates	SE	Estimates	SE	Estimates	SE
Skipstadsand	2014	90.50	4.26 ***	10.90	0.68 ***	30.61	2.02 ***
	2016	-1.50	4.29	-3.31	0.91 **	10.85	2.11 ***
Skjellvik	2014	84.00	3.75 ***	11.40	1.93 ***	53.68	1.88 ***
	2016	4.35	5.43	0.57	2.19	13.78	2.01 ***
Teneskjær	2014	92.20	4.48 ***	3.60	0.33 ***	15.78	4.14 **
	2016	-14.20	5.10 *	-0.66	0.35 .	18.25	3.39 ***

Table 3: Estimates and standard errors (SE) from the mixed models, investigating differences between locations in vegetation cover, vegetation height and soil moisture content in 2016. Skipstadsand is the reference location. Stars (*) indicates p-values from the mixed models, *** p<0.001; ** p<0.01; * p<0.05; . p<0.10. For pair-wise differences, see Appendix II.

Locations	**Vegetation cover (%)**		**Vegetation height (cm)**		**Soil moisture content (%)**	
	Estimates	SE	Estimates	SE	Estimates	SE
Skipstadsand	89.00	4.37 ***	7.59	1.17 ***	41.46	2.85 ***
Skjellvik	1.26	5.40	3.04	1.44 *	24.86	3.52 ***
Teneskjær	-11.00	6.18 .	-4.65	1.65 **	-7.43	4.03 .

PLANT PERFORMANCE OF MUSK ORCHID

In Skipstadsand the density of plants was higher in 2015 than 2014 (Fig 8 and see appendix III), but there was no significant difference between 2014 and 2016, and 2015 and 2016. No significant differences were found in plant density for Skjellvik and Teneskjær between the years (Fig 8 and see appendix III).

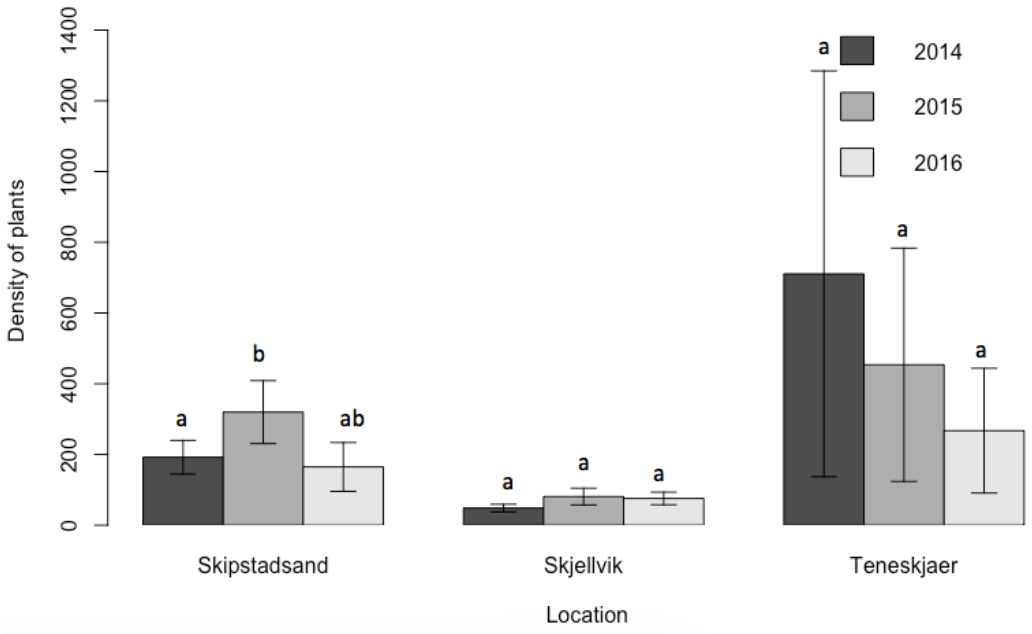

Figure 8 Density of musk orchid for the three locations. Different letters above the bar graphs indicates difference, and same letters means no significant difference, where each of the locations are tested separately in post-hoc Tukey tests.

At Skipstadsand the plant size was the largest in 2016 (Fig 9 and see appendix III), but no significant difference was found between 2014 and 2015. Also in Teneskjær the largest plant size was in 2016 (Fig 9 and see appendix III), where it was larger than in 2015. There was no difference between 2016 and 2014, and 2015 and 2014. This variation in Teneskjær however was not reflected in figure 9, as the density of plants varied between years, and this figure does not consider the variation in density and its effect on plant size. However, Skjellvik showed no significant difference between 2016 and 2014/2015. The plant size at Skjellvik was the lowest in 2015 (Fig 9 and see appendix III), where it was lower than in 2014.

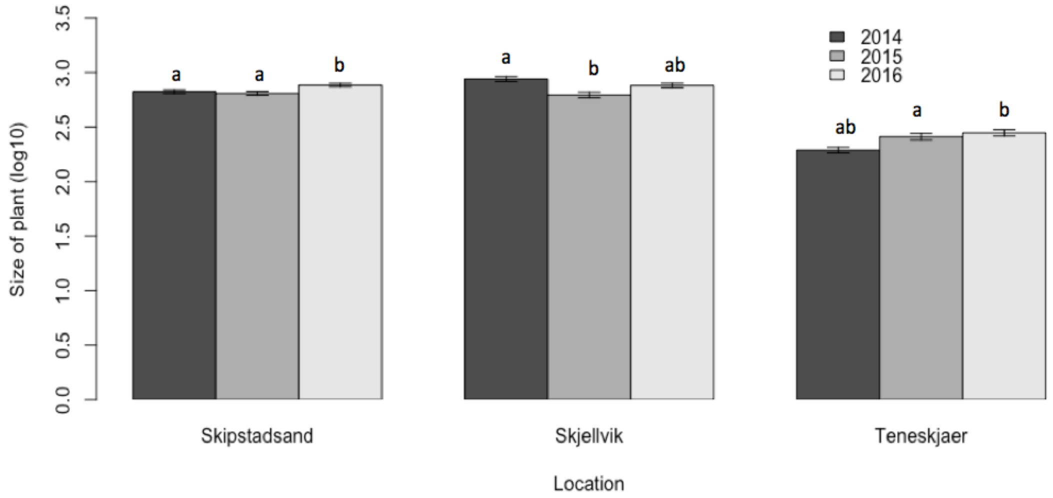

Figure 9 Size of the musk orchid for the three locations. Different letters above the bar graphs indicates difference, and same letters means no significant difference, where each of the locations are tested separately in post-hoc Tukey tests.

At Skipstadsand and Skjellvik the highest percentage of fertile plants were in 2014, there was no significant difference between 2015 and 2016 (Fig 10 and see appendix, table 8). In Teneskjær however there was no significant difference between the years.

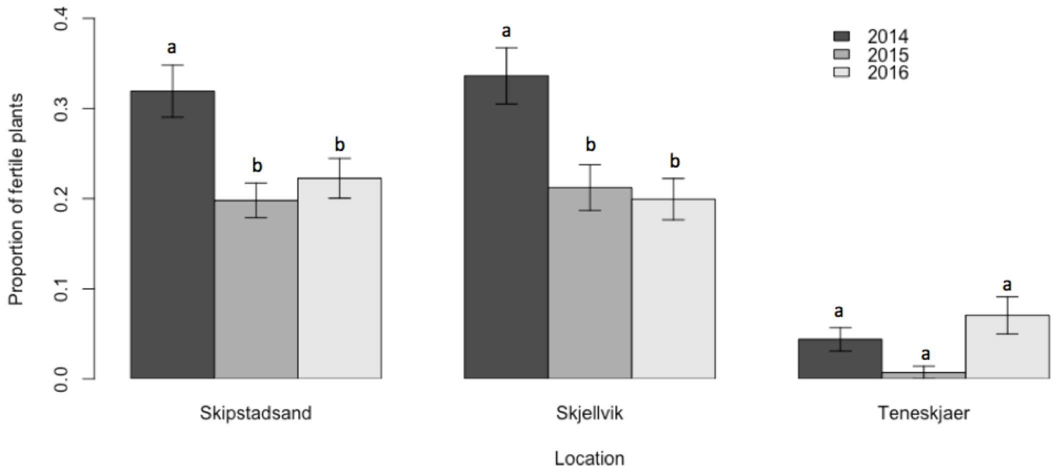

Figure 10 Proportion of fertile plants for the three locations between 2014 and 2016. Different letters above the bar graphs indicates difference, and same letters means no significant difference, where each of the locations are tested separately in post-hoc Tukey tests.

In Skipstadsand the highest number of flowers per plant were in 2014 (Fig 11 and see appendix III), and there was no significant difference between 2015 and 2016. Also in Skjellvik the highest number of flowers per plant were in 2014, but only 2016 was significant lower than 2014 (Fig 11 and appendix III). In Teneskjær, no significant difference in number of flowers per plants were found between the years.

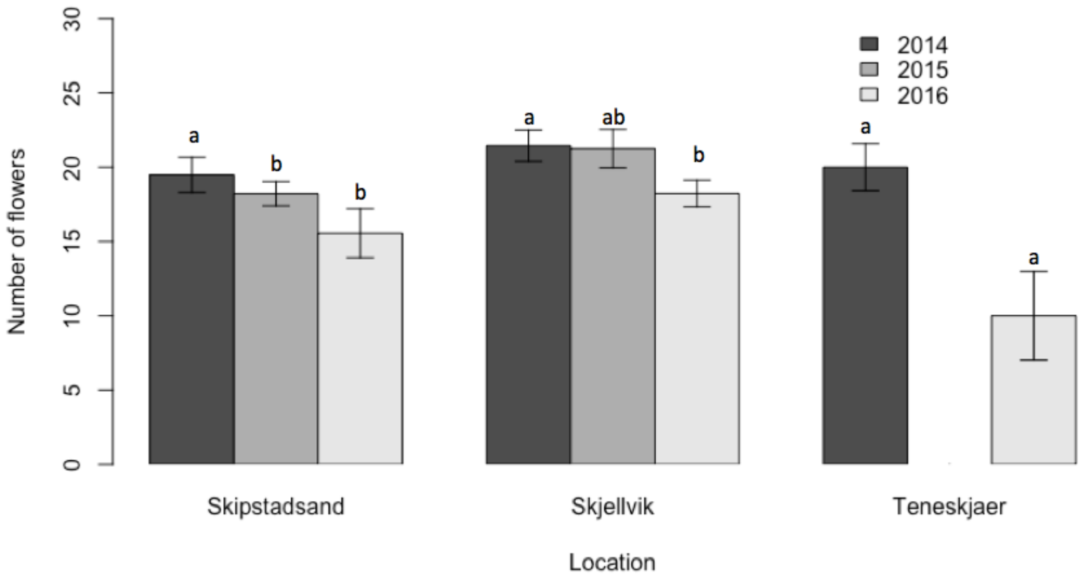

Figure 11 Number of flowers per plant for the three locations between 2014 and 2016. Different letters above the bar graphs indicates difference, and same letters means no significant difference, where each of the locations are tested separately in post-hoc Tukey tests.

GRAZING EFFECT ON MUSK ORCHID IN SKJELLVIK

No significant difference was found between the two sub-populations in any of the environmental factors (table 4 and 5).

Table 4 Mean and standard deviation (SD) for the environmental factors between the inside- and outside of the fence sub-populations at Skjellvik.

Sub-population	**Vegetation cover (%)**		**Vegetation height (cm)**		**Soil moisture content (%)**	
	Mean	SD	Mean	SD	Mean	SD
Inside of fence	82.5	13.79	9.53	4.25	61.285	7.99
Outside of fence	90.0	7.91	14.25	6.78	58.889	10.99

Table 5 Estimates and standard errors (SE) for the environmental factors for the two sub-populations at Skjellvik. Vegetation cover, vegetation height and soil moisture content were examined in mixed models. The outside of fence sub-population is the reference population. Written p-values are from post-hoc Tukey tests, the stars (*) indicate p-values from the mixed models, *** p<0.001; ** p<0.01; * p<0.05; . p<0.10.

Sub-population	**Vegetation cover (%)**		**Vegetation height (cm)**		**Soil moisture content (%)**	
	Estimates	SE	Estimates	SE	Estimates	SE
Outside of fence	90.00	3.80 ***	13.96	2.19 ***	58.90	3.09 ***
Inside of fence	-7.50	5.24	-4.44	3.04	2.40	4.27

Both the size of the plant and the proportion of fertile plants were significant larger in the sub-population outside of the fence at Skjellvik. There was no significant difference between the two sub-populations according number of flowers per plant and the density of musk orchid in the plots (Fig 12).

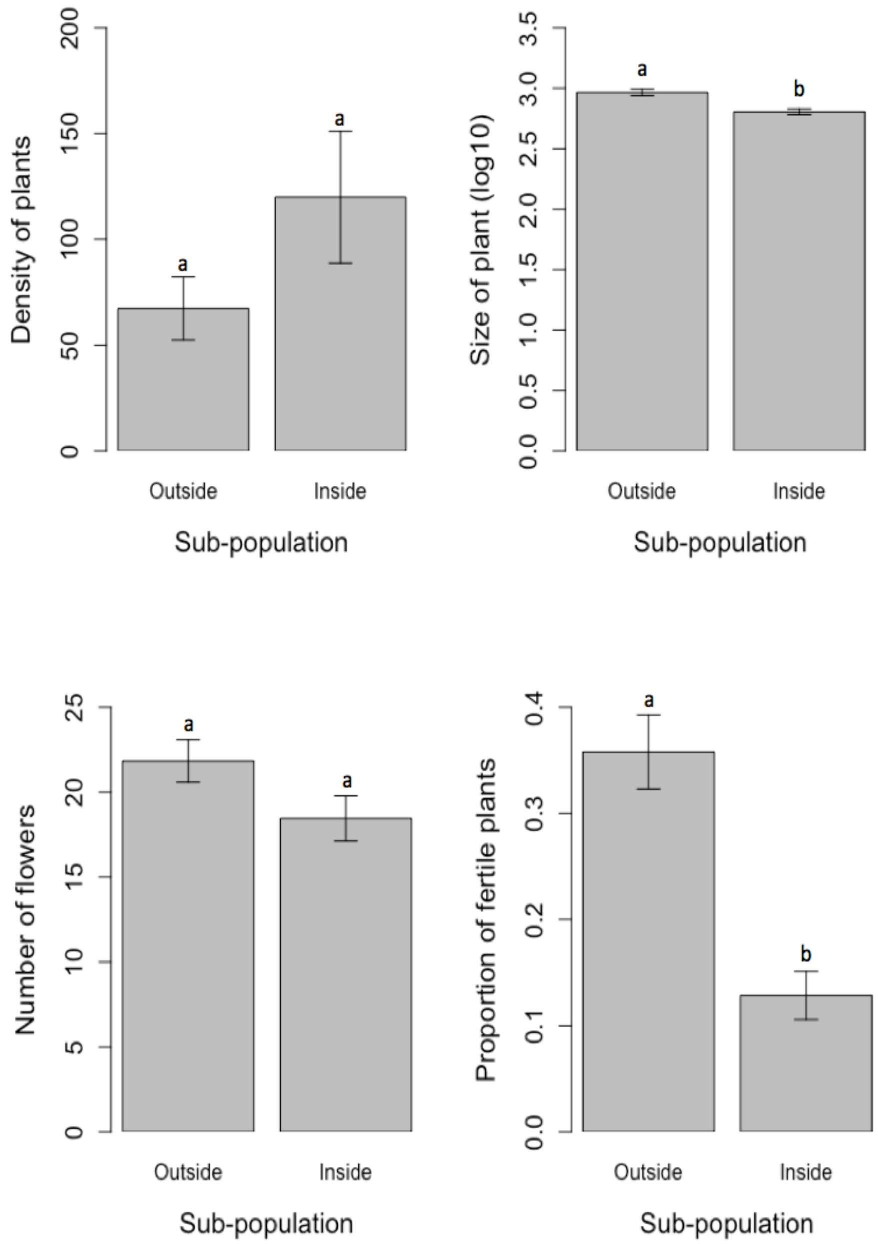

Figure 12 Difference between the two sub-populations when it comes to the plant variables density of plants, plant size, number of flowers and proportion of fertile plants. Different letters above the bar graphs indicates difference, and same letters means no significant difference, each of the plant variables were tested separately in post-hoc Tukey tests.

DISCUSSION

The main goal of this thesis is to get a better understanding of how the musk orchid is performing at Hvaler. To answer this I have addressed the three themes in my thesis, i.e. how the environment factors differ between the years and locations, the population performance of the musk orchid at the three locations separately over the three years, and how livestock grazing affect the musk orchid.

INTERACTION BETWEEN ENVIRONMENTAL FACTORS AND IN THE PERFORMANCE OF THE MUSK ORCHID

The soil moisture content in all the locations were clearly higher in 2016 as compared with 2014, which are well correlated with the climate report (Meteorologisk institutt 2017a). According to Meteorologisk institutt (2017a) the heaviest rainfall was in 2015, then followed by 2016 and 2014. This again affected the soil in the three locations, but maybe the most in Teneskjær, as there is a shallow soil depth here. The shallow soil depth will get a high soil moisture content shortly after rain, then shortly followed by dry soil due to water evaporating and taken up by the vegetation growing here.

As for the vegetation height, Skipstadsand was the only location that had a clear difference between 2016 and 2014. This can nevertheless be explained by cutting of Skipstadsand right before the fieldwork in 2016, as a mean to control meadowsweet (Norwegian: mjødurt) in the meadow.

The musk orchid individuals in Skjellvik were large, had a higher fertility rate and number of flowers than Teneskjær, suggesting that the musk orchid thrives the best in the wettest location. The low proportion of fertile plants in Teneskjær might be linked to the small leaf size, which has found to be a threshold for flowering in other orchids (Jacquemyn et al. 2007; Jacquemyn et al. 2010; Primack & Stacy 1998; Willems & Melser 1998). Wells et al. (1998) found that precipitation has a role in next year’s flowering in musk orchid, he also found that temperature has an impact.

The combining of shallow soil, dry soil and grazing livestock make the surrounding vegetation and the musk orchid small it Teneskjær, and make it visible that the area has several plant stressors. To compare with, Skjellvik and Skipstadsand had larger individuals, higher proportion of fertile plants as well as moister soil than Teneskjær. The vegetation surrounding the musk orchid also was more luscious. So, the soil moisture content has a great deal to say about the condition not only for the musk orchid, but also the surrounding plant community.

The higher density of musk orchid at Teneskjær might be because of the low surrounding vegetation and tramping interference from cattle which has made it possible for musk orchid seeds to germinate and clonally spreading to occur. To compare with the wettest location, Skjellvik, the density of musk orchid is the lowest. Probably due to the high surrounding vegetation, which grow too dense around the musk orchid, since there are no grazing there at the moment opens the vegetation, this will also affect the possibility for the musk orchid to spread either by seed or clonally.

Variation in plant density may be due to the mycorrhiza content in the ground. The higher mycorrhizal fungi near parent plants might induce higher recruitment near other adult individuals of musk orchid (De Hert et al. 2013; McCormick et al. 2016). The low surrounding vegetation and tramping interference from cattle that has made it possible for musk orchid to either spread clonally or with seeds, and might be the reason why there are high density at some of the plots in Teneskjær. However, seed recruitment in musk orchid is low, and the musk orchid spread therefore the most clonally (Miljødirektoratet 2010; Rasmussen 1995; Wells et al. 1998).

When conducting population analyses, it is most desirable to follow the analysis on an individual level. As we get better information about population size and development. I did not use the data on individual musk orchid in my study, and it is therefore a weakness in my study. I was not certain about the markers I placed next to the observed musk orchid, as the musk orchid does not emerge from the same place at the root tuber, in addition to growing densely at some of the locations. Therefore, last year's marked musk orchid can be; the marked individual, a new individual emerging nearer the marker, or a neighboring individual. For this reason, it has been especially hard to separate the individuals in the most densely growing areas; i.e. at some of the plots at Teneskjær. A second-best practice is to monitor groups of individuals in a defined area over several years, to be able to count both aboveground and dormant individuals. Which is the method I used in my analyses.

GRAZING EFFECT

It is clear that the musk orchid is affected by grazing, as there was a difference in both size and proportion of fertile plants between the sub-populations. The sub-population inside of the fence had a smaller proportion of fertile plants as well as being smaller in size, even though both locations where the sub-populations grew had no difference in the environmental factors. This indicate that the grazing effect has a long-term influence on the musk orchid. However, my results don't necessarily mean that grazing make the musk orchid grow larger. The lack of smaller individuals might suggest that the tramping from livestock has reduced the survival of younger individuals here, or remains from dung still fertilizes the area and make the individuals larger. Johansen et al. (2016) found an opposite trend for *Knautia arvensis* (Norwegian: rødknapp), also a perennial species associated with pastures, where the individuals that were excluded from grazing for a long time had higher proportion of fertile plants than in areas that where grazed at a low intensity.

These results correlates with the fact that the musk orchid relates to long-time managed pastures, and therefore will be best conserved in these kinds of managed areas (Ekstam & Forshed 1992). Livestock affects the vegetation by grazing and browsing, tramping damage and soil compaction, fertilization by dung and spreading of seeds (Norderhaug et al. 1999; Staaland et al. 1998b).

MANAGEMENT RECOMMENDATIONS

Monitoring is a crucial part of management, and three years of observation is not sufficient to tell how the performance of the musk orchid is. This is since we have only observed the above ground population, and there might be individuals that has stayed the last three years in the ground. Primack and Stacy (1998) suggest that studies with a duration of 4 to 7 years might be optimal to detect the cost of reproduction for perennial herbs, and therefore detect individuals that has stayed in a dormant stage the first years of the study.

An important discussion around management of the musk orchid is whether the populations should be grazed or hayed, and which livestock type that is most suitable. Rostad (2016) discusses if cattle are the wrong type of livestock, and that goats might be a better fit as they are browsers and not grazers as compared cattle. This might help keeping the woody species down, as one of the main threat toward the musk orchid is overgrowing, however goats also tend to favor herbs (Bryn 2001; Miljødirektoratet 2010; Staaland et al. 1998b; Stusdal 2006). The gentlest forager in herb dominated vegetation is cattle, as they forage evenly and do little sorting (Norderhaug et al. 1999).

Historically cattle have been the livestock grazing at Hvaler, and therefore the vegetation here is formed around grazing management (Miljødirektoratet 2010). According to Miljødirektoratet (2010) were the light cow races East-Norwegian reddish (Norwegian: Østnorsk Rødkolle) and Telemark cattle (Norwegian: Telemarksku) the cattle type that grazed the area prior to 1950, today the cattle type is Norwegian red (Norwegian: NRF / Norsk Rødt Fe). To compare, an adult NRF cow can become up to 230 kg heavier, and an adult NRF bull, up to 650 kg heavier than the two older races (Rundlöf 2014). However, to reintroduce grazing in Skjellvik is risky, as the soil at this location moist and easily disturbed by the cattle tramping. So before reintroducing a smaller cow race or lower density of cattle, it would be beneficial to try the cattle race or density in areas at Skjellvik where the musk orchid is not growing. To determine if it has a lower tramping effect on the ground than the current cattle race and density.

A concern with only haying the meadows without no livestock, is that the vegetation might grow to dense (Johansen et al. 2016; Miljødirektoratet 2010; Norderhaug et al. 1999). Haying practices are also considered a heavy and tedious management regime, which are expensive to execute (Direktoratet for naturforvaltning 2009). Livestock, such as cattle make gaps that opens the vegetation and it is will be possible for the musk orchid to recruit. Too many or too large livestock however will make this effect too extreme, and it will become a problem instead of (Bryn 2001; Stusdal 2006).

CONCLUSION

Long time monitoring of musk orchid, or other perennial species with dormant stages, are crucial in population analyses. After three years, it is still a bit early to tell how the musk orchid populations are performing at Hvaler, as it is uncertain how long the musk orchid can stay in the dormant stage. I will therefore recommend continuing the monitoring a couple of years, to get a better picture of the whole population.

From my results, it seems that the moisture content in the soil has clearly a positive impact not only on the performance of the musk orchid, but the surrounding vegetation as well. And without grazing to open the vegetation, water content will work against the musk orchid, as the surrounding vegetation create too much competition. It can also be beneficial to include more abiotic environmental factors in future studies; i.e. salinity, fertilization components such as nitrogen, and mycorrhiza fungi content in the soil, to detect potential differences between the locations.

I believe grazing is an important management mean as it keeps vegetation down as well as make openings in the dense vegetation. As long it is done with the right density of livestock in addition to the right type and size of livestock. However, it is important to exclude grazing around the musk orchid populations while it's in the fertile stage.

In 2016, there was found a new musk orchid location the island Filletassen, an island between Asmaløy and Kirkeøy (Høitomt & Brynjulvsrud 2017). Since the musk orchid is a species that can easily be over looked as it has a discreet appearance, there might be more musk orchid populations in both Hvaler municipality and elsewhere in Norway. This new finding brightens the future for the musk orchid in Norway.

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APPENDIX I

The p-values from the mixed models, where the environmental factors recorded in 2014 and 2016 were tested to examine differences between years. for each location, separately.

Location	Vegetation cover	Vegetation height	Soil moisture content
Skipstadsand	0.726	0.001	0.001
Skjellvik	0.423	0,794	0.001
Teneskjær	0.005	0.055	0.001

APPENDIX II

The p-values from the post-hoc Tukey test, where the environmental factors were tested. The environmental factors were examined; i.e. vegetation cover, vegetation height and soil moisture content difference between the three locations in 2016.

Location	Vegetation cover	Vegetation height	Soil moisture content
Skjellvik - Skipstadsand	0.970	0.088	0.001
Teneskjær – Skipstadsand	0.175	0.013	0.099
Teneskjær – Skjellvik	0.059	0.001	0.001

APPENDIX III

The p-values from the post-hoc Tukey test, where the plant variables were tested for each location separately. The density of plants, biomass, number of flowers and if flowering occurred were examined to look for difference between the three years.

Location	Year	Density of plants	Size of plants	Flowering	Flowers
Skipstadsand	2015- 2014	0.005	0.833	0.001	0.003
	2016- 2014	0.355	0.048	0,004	0.001
	2016- 2015	0.215	0.003	0.862	0.262
Skjellvik	2015- 2014	0.077	0.001	0.020	0.169
	2016- 2014	0.067	0.058	0.014	0.001
	2016- 2015	0.989	0.157	0.997	0.142
Teneskjær	2015- 2014	0.641	0.305	0.065	0.108
	2016- 2014	0.268	0.229	0.993	0.912
	2016- 2015	0.792	0.012	0.057	0.271

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