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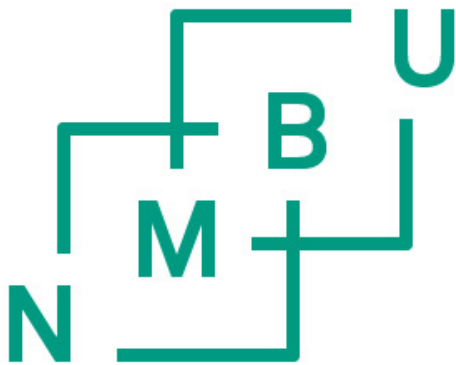
Department for Ecology and Natural Resource Management

The Effects of Changes in Livestock Grazing and Agricultural Land Use on an Endangered Orchid, *Herminium monorchis*, in Norway; a Correlative and Experimental Approach

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Abstract

Changes in agricultural practices and land use, with abandonment of low-intensity grazing and the transformation of semi-natural meadows into cultivated agricultural areas, have shown negative impacts on several plant species and communities across Europe. These changes are likely to have driven many species, such as orchids, close to local and regional extinction. In this study, I investigate reproductive output and growth responses of *Herminium monorchis*, a critically endangered orchid species in Norway, to experimental grazing. Because livestock grazing may alter plant densities, I also examine the relation of reproduction to conspecific plant density and floral display. Additionally, I compare the historical abundance of *H. monorchis* populations in Norway to historical data of grazing livestock densities and the change in area of semi-natural mown meadows. I found that *H. monorchis* set fewer fruits per flower but grows taller under experimental grazing. Reproduction was not related to conspecific plant density or floral display. Furthermore, the number of fruits set was positively related to soil salinity, which is surprising since very few orchids are known to be halophytes. The historical data show that the number of *H. monorchis* populations has decreased significantly since 1907, and this decrease was best explained by a reduction in the number of goats and a reduction in the area of semi-natural mown meadows. My results suggest that the Norwegian population of *H. monorchis* should not be exposed to livestock grazing during the fertile reproductive stage. The positive relation of *H. monorchis* populations to natural mown area and number of goats suggests managing the extant populations or establishing new by applying mowing of vegetation and grazing by goats.

Sammendrag

Endringer av driftsformer og arealbruk i landbruket, i form av mindre lav-intensitetsbeiting og omgjøring av semi-naturlig slåtte- og beitemark til kultiverte landbruksarealer, har vist negative påvirkninger på flere plantearter og plantesamfunn i Europa. Disse endringene ser ut til å ha drevet mange plantearter, som orkidéer, nær lokal eller regional utryddelse. I dette studiet undersøker jeg hvordan reproduksjon og vekst hos honningblom (*Herminium monorchis*), en kritisk truet orkidé i Norge, påvirkes av eksperimentell beiting. Beiting fra husdyr kan påvirke plantetetthet, derfor ser jeg på hvordan reproduksjon hos honningblom er tilknyttet tetthet av artsfrender og antall blomster per plante. I tillegg sammenligner jeg antall populasjoner av honningblom med historiske data på antall beitedyr og arealmessig endring i semi-naturlig slåttemark. Jeg fant ut at honningblom produserer færre frukter per blomst, men vokser høyere under eksperimentell beiting. Reproduksjon hadde ingen sammenheng med plantetetthet eller antall blomster. Videre fant jeg at antall frukt per blomst var positivt tilknyttet konsentrasjonen av salt i jorden, noe som er overraskende siden svært få orkidéer er kjent som halofytter. De historiske dataene viser at antall populasjoner av honningblom har sunket signifikant siden 1907. Denne nedgangen ble best forklart av nedgang i antall geiter og areal med semi-naturlig slåttemark. Mine resultater tilsier at bruk av beitedyr for å fremme honningblom i Norge kan virke mot sin hensikt om beiting skjer i plantens reproduktive stadium. Om beiting skal brukes som tiltak for å fremme honningblom i Norge, bør honningblomindividene skånes for beitedyr i den tiden på året plantene er sitt fertile stadium og setter blomsterstand. Sammenhengen honningblompopulasjoner har vist med areal naturlig slåttemark og antall geiter, foreslår at allerede eksisterende eller potensielt nye populasjoner av honningblom i Norge bør legges under forvaltningstiltak som mekanisk slått eller beitereregimer med geit.

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1. INTRODUCTION

Human land use appears as one of the most important global change stressors on ecosystems (Vitousek et al., 1997b). Across Europe there is an on-going reduction in the extent of low-intensity farming systems, with the abandonment of low intensity grazing systems in unfenced and uncultivated areas as a response to the increased practice of keeping livestock in fenced enclosures or in housings (Beaufoy et al., 1994). Grazing affects the composition of plant functional traits, morphology and phenology (Briske, 1996), as it operates as an important disturbance factor in plant communities and ecosystems (Huntly, 1991), and many plant species show a positive abundance response to this (Diaz et al., 2007). Thus, abandonment or change in the management of grasslands may influence the composition of plant functional traits, and species richness and species composition, with different magnitudes in different areas (Bakker, 1989, Bakker et al., 2002, Kahmen et al., 2002, Moog et al., 2002, Kahmen and Poschlod, 2004). In Norway, the amount of grazing livestock and the practice of hay mowing in unfertilized areas has decreased significantly during the last century (SSB, 1907-1999), which is suggested by Bryn (2001) to have an impact on plant diversity and the decline of many Norwegian plant species occurring in grazed and mown vegetation types.

Orchidaceae is one of the largest plant families, with an estimate of more than 25 000 species globally (Cribb et al., 2003). Among plants, Orchids is plant family with the highest proportion of threatened species in the world, mainly due to habitat loss and degradation, changes in grazing pressure and loss of pollinators (Swarts and Dixon, 2009). Orchids are also showing a decline across Europe, especially species preferring calcareous grassland (Kull and Hutchings, 2006). In Norway, orchids is the plant group with the largest proportion of threatened plant species, with 21 of 40 species being listed on the Norwegian Red List of Species (Henriksen and Hilmo, 2015). Most of these species occur in natural or semi-natural grasslands (Mossberg et al., 2012), which are vegetation types that have decreased in Norway due to changes in agricultural land use practices. This is believed to be the reason for the large proportion of threatened orchid species in Norway. Furthermore, some orchid species have been shown to suffer from taller surrounding vegetation, due to changes in animal grazing and anthropogenic land use (Tali et al., 2004). For example, the orchid *Ophrys sphegodes* was shown to have recruitment exceeding mortality under sheep grazing regimes (Waite and Hutchings, 1991). Still, Hutchings (2010) suggests that grazing animals should be removed during flowering and seed set periods to mitigate the negative effects of grazing on orchid reproduction (Hutchings, 2010).

Individuals of many plant species may experience reduced reproductive output when they grow in small patches, in isolation, at low densities, or with the three latter conditions in combination (Groom, 1998). Larger and denser plant populations increase pollinator attractiveness and the amount of potential mates, and at the same time pollinators tend to spend more time and visit more flowers in larger populations, which in turn increases fruit set (Klinkhamer et al., 1989, Ågren, 1996, Ashman et al., 2004). Some studies have found that conspecific plant densities increases fruit set but not pollinator visitation rates (Lázaro et al., 2013). Pollinators have been shown to visit more flowers per plant under low plant densities, which may cause reduced outcrossing and increased selfing through geitogamous pollination (Field et al., 2005). Often plants have higher fitness when

growing in clumps, and they show higher fruit set in dense populations (Silander Jr, 1978). On the other hand, small and fragmented plant populations often suffer lower pollinator visitation and a subsequent lower fruit set (Jennersten, 1988). Orchids are generally pollen limited (Tremblay et al., 2005), which suggests that low pollinator availability or ineffective pollinators may constrain fruit production. For the orchid *Listera ovata* plants with many floral displays had more pollinia removed and higher fruit set, with reproductive success generally positively correlated with population size. But pollinia removal was little affected by population size, which suggests that higher fruit set cannot be explained by increased pollinator visits alone, but rather that pollinators are more likely to visit another orchid if the population size is large (Brys et al., 2008). Thus, one would expect rare orchids to be especially vulnerable when occurring in small, fragmented populations, perhaps suffering a negative feedback where small populations attract fewer pollinators and set less fruit, resulting in an even lower population size the following years. Nevertheless, small population size in some orchids may show a negative, positive or no relationship with pollinator visitation rate, so the effect of small, fragmented populations on pollinator visitation is difficult to predict.

Herminium monorchis is perhaps the rarest orchid in Norway, occurring only with three populations (Båtvik 2010), and it is placed in the category *Critically endangered (CR)* on The 2010 Norwegian red list of species (Henriksen and Hilmo, 2015). It appears to have a low tolerance for competition and nutrient rich conditions, and the decline is suggested to be caused by land use changes, such as reduced livestock grazing and increased urban development (Båtvik, 2010). Cattle have historically been grazing in several of the now extinct *H. monorchis* populations, but this practice has ceased in most of the locations, making it a possible reason for the species decline (Båtvik, 2010). *H. monorchis* also occur under grazing regimes by other mammals, like sheep and rabbits (Wells et al., 1998). The three last *H. monorchis* locations in Norway are currently not grazed, but woody vegetation is managed and kept low by mechanical mowing. *H. monorchis* is also known tolerate saline conditions (Økland, 1996), and the current populations of *H. monorchis* in Norway occur very close to the sea, where they are directly exposed to salt water spray.

Nectar-producing orchids tend to set fruit more successfully than unrewarding species (Neiland and Wilcock, 1998), and this also applies for *H. monorchis*, which has a relative high seed production compared to other nectar-producing orchids (Nilsson, 1979). Nilsson (1979) also revealed that the plant attracts a wide array of pollinator species from Coleoptera, Diptera and Hymenoptera, and this is unusual for orchids which normally are visited by few pollinator species (Schiestl and Schlüter, 2009). Field experiments on the orchid species *Caladenia arenicola* have shown that a very low proportion of seeds germinate in the wild (1%), and that even fewer seedlings reach a stage where they can develop in to a viable plant (0.0013 %) (Batty et al., 2001). These numbers have been used to estimate potential recruitment in another orchid species (*Caladenia rigida*), where they calculated the seedling recruitment in a small population to be lower than individual survival and insufficient to ensure long-term population survival (Faast et al., 2011). This could also apply for *H. monorchis* in Norway, as the last remaining populations are small in size and occur on little area. Additionally, a low seed production in the populations could also limit seed dispersal and with a subsequent lower chance to establish populations in new areas. Thus, revealing the effects of livestock grazing on the reproduction of *H. monorchis* could be important for the conservation of this species in Norway.

In this study I examine the decrease of *H. monorchis* by assessing historical data of *H. monorchis* and agricultural land use. I also conducted experimental grazing of fertile individuals in an extant *H. monorchis* population to understand how grazing potentially affects its reproductive output, plant size and growth. Because conspecific density may have different effects on orchid reproduction, and that grazing livestock possibly can alter conspecific density, I wish to examine the relations between reproductive output and conspecific plant density at patch level and floral display per inflorescence in this population. I also want to reveal how *H. monorchis* reacts to different soil conditions. In particular I ask the following questions:

1. Is the population decline of *H. monorchis* in Norway related to historical changes in agricultural land use and livestock density?
2. Does experimental livestock grazing during the fertile stage of *H. monorchis* affect reproductive output, size and growth?
3. Does conspecific plant density and floral display explain *H. monorchis* reproductive output, size and growth?
4. How is reproduction and growth in *H. monorchis* related to micro-site soil conditions?

2. MATERIALS AND METHODS

2.1. STUDY AREA AND STUDY SPECIES

I conducted the study in one of the three known Norwegian populations of *H. monorchis* at Skjellvik (59°03'14"N 10°55'26"E) on the island Asmaløy in Hvaler municipality, Østfold County. With an area of 9.1 km², this is a fairly large island in the Hvaler archipelago, where it is connected to the mainland through a series of islands and both ground level and elevated bridges.

H. monorchis is a small-sized orchid, reaching a height of ca 7-25 cm. It has two to three 20-70 x 3-10 mm large leaves at ground level, and a few smaller leaves on the stalk. The flowers are yellow, honey scented and small (2.5-4 mm long perianth and 3.5-4 mm long labellum) (Tutin, 1980). *H. monorchis* may not become fertile and set inflorescence every year, and may in some years remain in a vegetative stage only (Wells et al., 1998).

In Scandinavia, *H. monorchis* occurs on calcareous and humid soil in pastures, meadows, beaches and fens (Mossberg et al., 2012). The species is also known to tolerate saline conditions (Økland, 1996), and all of the three Norwegian populations occur close to the sea, with one of them being within direct proximity of the ocean where it is exposed to saltwater spray (Båtvik, 2010). The focal population in this study (Figure 1) grows on extremely rich fen where I measured soil pH to range between 6 and 7.4 and soil water content to range from 75.15 % to 93.35 % (Fremstad, 1997).

Because of its population decline and apparent loss of habitat to land use change, *H. monorchis* should make a good species to monitor when investigating the relationship between declining plant species and changes in human land use.

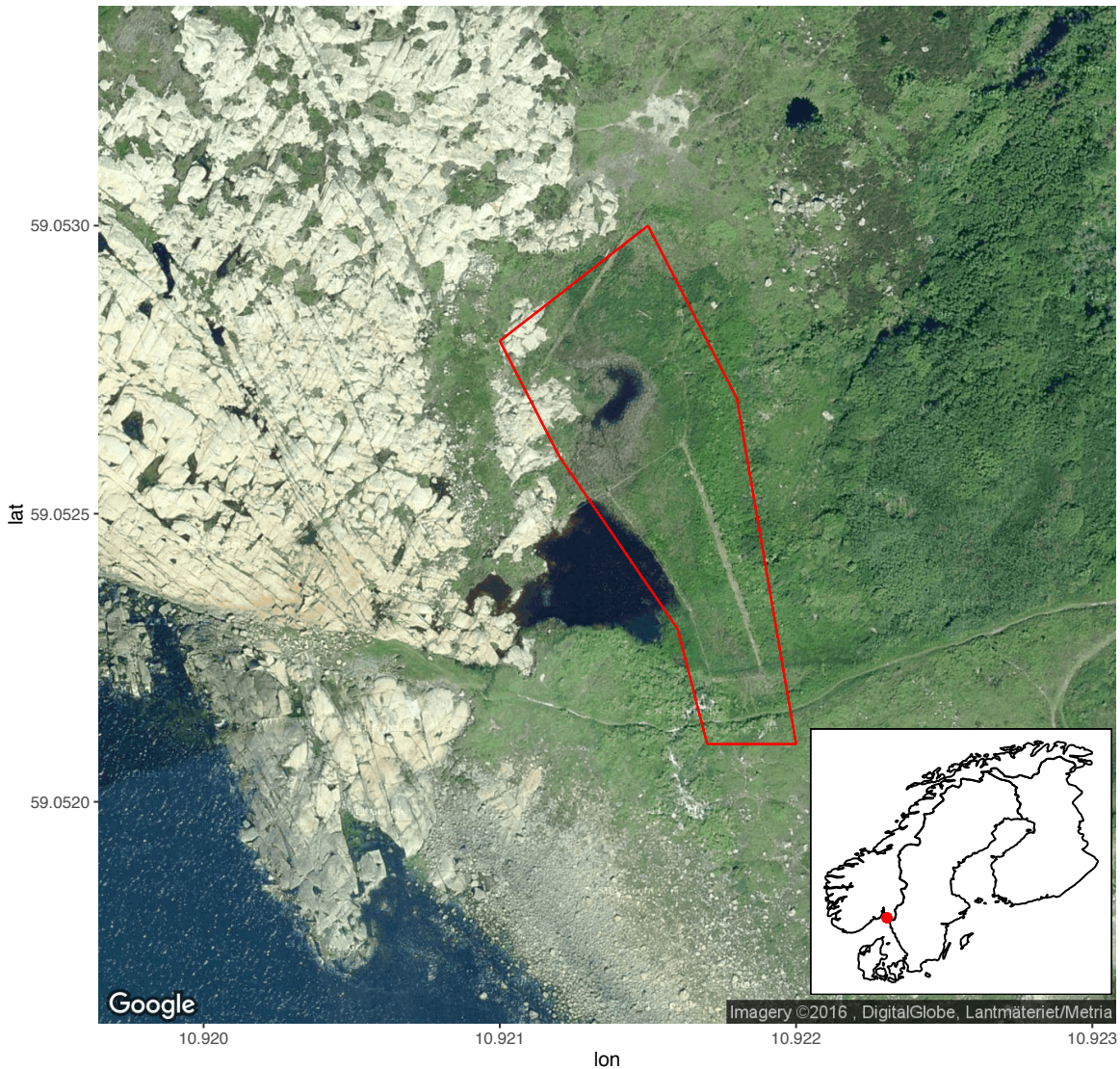


Figure 1. Map of the study area. The red dot marks the study location on Asmaløy in Norway, and the red polygon marks the area where *Herminium monorchis* occur in the location.

2.2. DATA COLLECTION

2.2.1 Historical data

In order to examine if the decrease in Norwegian *H. monorchis* populations is related to changes in Norwegian agriculture, I collected historical data of land use and livestock practices in Norwegian agriculture from the Norwegian agricultural censuses, which has been conducted approximately every tenth year from 1907 to 1999 (SSB, 1907-1999). These data were collected at a) both the whole country level and b) at a smaller level, including only counties where *H. monorchis* has occurred historically. The analysis of data a) and b) gave very similar results, and I decided to only present results from changes on country level to reflect the changes in all of Norway.

Changes in land use

I did not assess changes in the amount of grazed area in the statistics because the definitions of natural and cultivated grazed areas have changed several times in the agricultural censuses, which made the data too inconsistent for statistical analysis. Instead, I assessed the changes in areas for

hay mowing into two categories: a) mown natural meadows (with no or minimal fertilization, but with some surface management), and b) mown cultivated meadows (regularly tilled and fertilized)

Changes in livestock numbers

From the 1907-1999 database, I assessed the number of grazing livestock, including goats, cattle, sheep and horses.

***H. monorchis* populations**

Båtvik (2010) used the collection date and collection location of dried herbarium specimens of *H. monorchis* to assess where and at what time *H. monorchis* populations have been found in Norway during the period 1812-2008. To obtain an estimate of the historical change in the number of populations of *H. monorchis* I counted the number of populations where specimens had been collected from at the same time or after the year of each agricultural count from 1907-1999. This did not take into account that new populations may have been established and that they may have added up to the earlier population counts.

2.2.2. Effects of removal of vegetation on reproductive success and growth

In order to examine experimentally the effects of grazing on reproductive success and growth I established a randomized block experiment with thirty blocks that each contained two fertile plants with inflorescence, totalling sixty plants. I selected the blocks randomly within the study area (Figure 1) by walking four parallel lines from south to north across the study area and blindly throwing an object behind my back for each tenth step. The focal block was then established around the two orchids occurring closest to the landing site of the thrown object. A 25 x 25 cm quadrat was marked around both plants in each block, with no more than 94 cm between each plant, and each quadrat tagged with block number and treatment type. Each quadrat was oriented similarly after cardinal directions. For each block, one quadrat was manipulated by cutting all vegetation, (except *H. monorchis*) to ground level and by keeping the vegetation low throughout the season in an attempt to simulate livestock grazing, whereas the vegetation in the other quadrat was left uncut as a control. The blocks were selected on the 29th of June 2015, and vegetation was removed from the quadrats between 29th of June 2015 and 1st of July 2015 and kept low until 28th of August 2015.

2.2.3. Conspecific plant density and floral display

To measure conspecific plant densities I counted all *H. monorchis* inflorescences occurring within squares of 25 x 25 cm, 50 x 50 cm, 100 x 100 cm and 200 x 200 cm around the focal plants from the grazing experiment. Furthermore, I measured floral display (number of flowers) for each focal inflorescence.

2.2.4 Reproduction, plant size and growth

Reproduction

I measured plant reproduction at the end (28th of August 2015) of the experiment. The number of fruits was counted, and fruit size (length x diameter) of the five lowermost positioned fruits of each plant was measured with a caliper after all successfully fertilized fruits on the inflorescences had ripened (28th of August 2015). Orchid fruit size has been shown to be an adequate estimate of seed number (Faast et al., 2011), thus I measured fruit size to get an approximate measure of seed production without damaging the plants.

Plant height

I measured the height of the plant from the bottom of the stalk to the top of the inflorescence with a metric ruler at the beginning (3rd of July 2015) and at the end of the experiment (28th of August).

Leaf area

I counted all leaves and measured leaf length (from basis of the leaf to the tip of the leaf) and leaf width (across the widest section of the leaf) for each leaf on each plant with a metric ruler at the beginning (3rd of July 2015) and the end (28th of August 2015) of the field experiment. Leaf length was multiplied with leaf width for each leaf and added up to the total leaf area for each plant.

2.3. SOIL SAMPLE ANALYSIS

I collected a small soil sample 3-4 cm deep in the surface soil layer below each focal plant, in order to reveal levels of salinity, organic matter content, soil water content and pH in the soil. The samples were collected as close to the plant as possible without damaging the plant. I froze the samples within two hours after sampling and kept them frozen until soil analysis to avoid microbial activity and water evaporation. The soil samples were gathered at the last day of the grazing experiment (29th of August 2015) to avoid disturbing the *H. monorchis* individuals before fruit maturation.

2.3.1. Soil water content

To estimate soil water content, I assigned each soil sample to unique numbered and weighed clay crucibles. A sufficient amount of wet soil (at least five gram) was added to each crucible before weighing them again. The wet soil was then dried overnight in a furnace at 105 °C, cooled in glass dessicators and weighed again. The difference in percent between wet weight and dry weight was used as a measure of soil water content.

2.3.2 Practical salinity and pH

To measure salinity and pH in the soil samples, I took weighed and dried soil from each sample and added 10 mL deionized water in a plastic tube, where it was mixed with a vortex shaker. The samples were kept refrigerated for 68 hours to allow the dry matter to be diluted in the water properly, before shaking them again immediately before measuring practical salinity and pH (see below). Then I used an electrical conductivity meter, which measures the concentration of dissolved ions, to measure electrical conductivity in microSiemens per cm³ for each soil sample. The conductivity values was divided by the amount of dry soil added in the test tubes and used as a measure of practical salinity of each soil sample.

The pH values of each soil sample were measured for each sample with a benchtop pH meter. The pH meter never settled, and the value was continuously sinking during measurement on individual samples. Therefore, I consistently noted the value only when the reading remained unchanged for five seconds.

2.3.3. Organic matter content

To determine organic matter content of the soil, I first weighed, and then incinerated the crucibles with the dried samples at 600 °C. The burned samples were placed in airtight glass dessicators during cooling to avoid water condensation from the air to the soil. The purpose of this process was to incinerate away all organic content and leave the mineral content only. After incineration, the

samples were weighed again, and the difference in percent between the dry weight and mineral weight was used as a measure of soil organic content.

2.4. STATISTICAL ANALYSIS

Only 56 of the initial 60 sampled orchids were used in the statistical analysis due to sampling error. The analysis of fruit size and plant size growth was done with the data on plants that kept their inflorescence to the end of the experiment ($n = 21$)(many inflorescences withered before reaching fruit maturity).

2.4.1. Historical data

For the number of *H. monorchis* populations, I fitted linear models with numbers of the different livestock species, and the area of mown natural and cultivated meadows as predictor variables. The models were selected by stepwise backward selection, with p-value as evaluation criterion.

2.4.2. Reproductive output, plant size, growth, conspecific density and floral display

I used mixed models (methods and background can be found in Bolker et al. (2009)) to reveal the effects of the manipulative experiment and the relation between plant size, plant growth, plant densities, floral display (number of flowers) and soil conditions on the number of fruits set, fruit volume, the probability to set fruit, initial plant height, initial leaf area, plant height growth and leaf area growth of the focal plants. I used block number as a random effect to adjust for variation between blocks. All models were selected on the Akaike Information Criterion (AIC) to approach the “true” model explaining the data (Burnham and Anderson, 2003). I selected the best models with the dredge function in the R package MuMIn (Bartoń, 2013), which automatically finds the combination of variables that gives a model with the lowest AIC value, and the variables in this “best” model may not necessarily have statistically significant relations to the response. Fruit volume and the plant and size growth variables were normal distributed and hence explained with a linear mixed model, and the probability to keep the inflorescence until fruit maturity was explained in a generalized mixed model with a binomial distribution. All these models were made in the R package lme4 (Bates et al., 2014). In order to explain fruit set, I fitted a generalized linear mixed model with flower amount as an offset variable. I used flowers as an offset term to adjust for the relation between initial flower amount and number of fruits. Due to overdispersion and an excess amount of zeroes in the fruit set data, I used a zero inflated negative binomial distribution (ZINB) in the models (Perumean-Chaney et al., 2013). Whether to use negative binomial distribution 1 or 2 was decided on the AIC values of the produced models. The GLMMs used to explain fruit set were made with the R package glmmADMB (Bolker et al., 2012).

2.4.3 Plant size and growth

I estimated total plant height growth and leaf area growth by using the last measured values as the response variable with the first measures as an offset variable in the statistical models. When using plant height growth and leaf area growth as explanatory variables in the figures, I used the ratio between last measured value and the initial value as a measure of growth.

3. RESULTS

3.1. HISTORICAL DATA

The amount of natural mown area and the number of goats has decreased significantly over time in Norway (Figure 2A&B). However, the decline in the number of *H. monorchis* populations is better explained by the amount of natural mown area (Figure 2D) than by year (Figure 2C), although the difference in explanatory power is very low. The number of goats explains the number of orchid populations quite well (Figure 2E) but lose explanatory power (p-value for the goat coefficient = 0.054) when the amount of mown natural area is taken into consideration. The number of *H. monorchis* populations was best explained by a model with number of goats and natural mown area as explanatory variables (linear regression model: $y = 1.54 + 0.003 * \text{mown natural area} + 0.0013 * \text{number of goats}$, $r^2 \text{ Adj.} = 0.95$, $p = 0.0006$). The number of orchid populations had no significant relationship with the number of cows, horses or with the area of cultivated mown meadows.

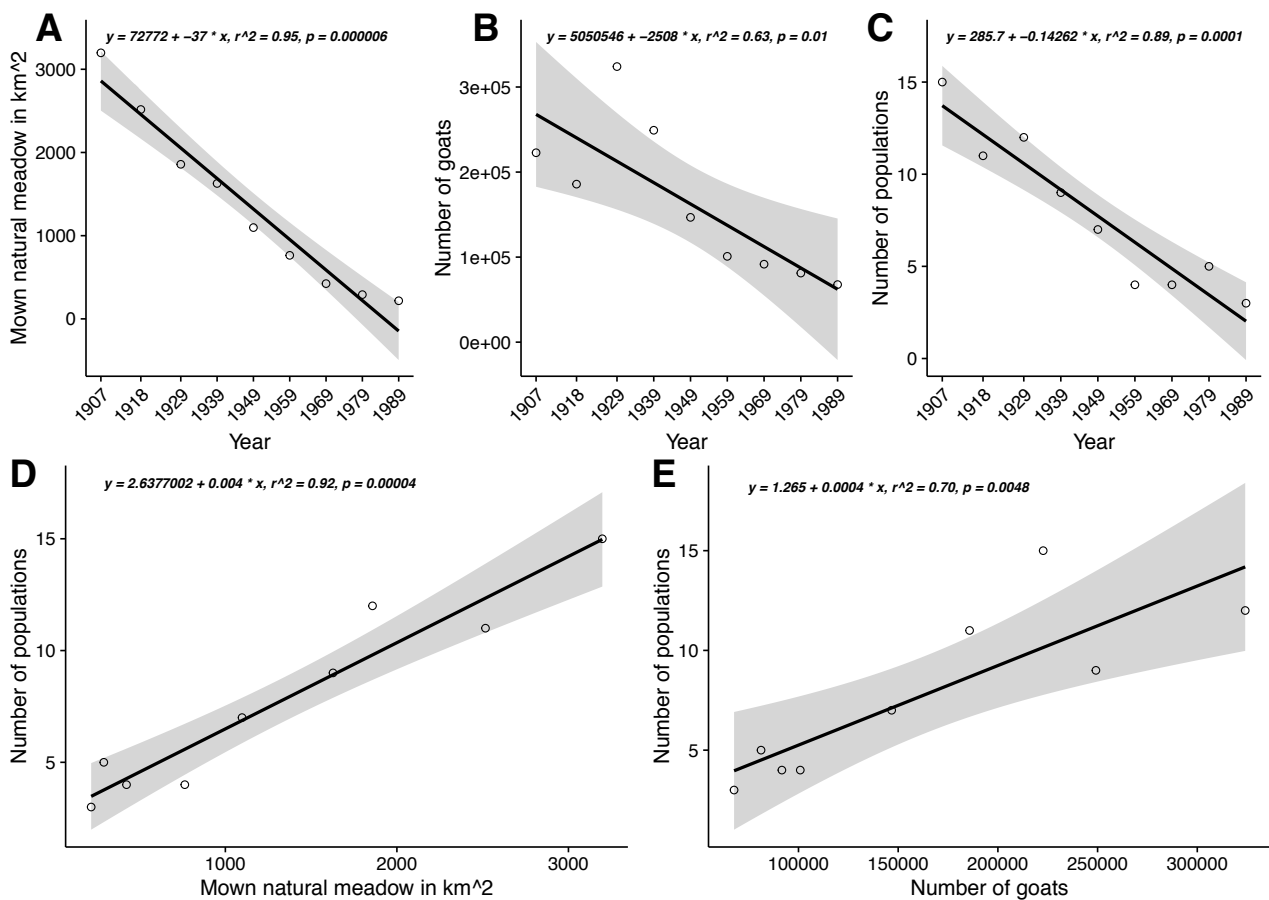


Figure 2. The changes in agricultural land use and the decrease of *Herminium monorchis* populations in Norway (A) The decrease in mown natural meadow from 1907-1989. (B) The decrease in number of goats from 1907-1989. (C) The decrease in number of *H. monorchis* populations from 1907-1989. (D) The relationship between *H. monorchis* populations and the area of mown natural meadow. (E) The relationship between *H. monorchis* populations and the number of goats.

3.2. VEGETATION REMOVAL

The experimental grazing, through removal of vegetation, had a significant negative effect on the number of fruits set and a non-significant relation to fruit size. It had no significant effect on the probability of a plant to keep its inflorescence to fruit maturation or on leaf area. Additionally, removal of vegetation had a significant positive effect ($p < 0.01$) on plant height growth. Vegetation removal and leaf growth had a significant positive interaction effect on fruit size (Table 1).

3.3. CONSPECIFIC DENSITY AND FLORAL DISPLAY

Conspecific plant density and floral display had no significant relationships to any response variable with any explanatory variable in any model.

3.4. PLANT SIZE AND GROWTH

The number of fruits set was significant negatively related to initial plant size, Additionally, there was a significant positive interaction effect between initial plant size and plant height growth, where the positive effect of plant growth increased with initial plant height. Number of fruits and fruit size showed a significant positive relationship with plant height growth, additionally, fruit size appeared to be related to a non-significant interaction effect between plant height growth and vegetation removal. On the other hand fruit size was significantly negatively related to leaf area growth, with leaf area growth showing a significant positive interaction with vegetation removal. Plant height growth was significantly positively related to the number of fruits set (Table 1).

3.5. SOIL CONDITIONS

The number of fruits and the probability for a plant to keep it inflorescence until fruit maturity (probability to set any fruit) showed a significant negative relationship to soil organic matter content, while initial leaf area and leaf area growth showed a non-significant relation to soil organic matter content. The number of fruits set and the probability to set any fruit showed a significant positive relationship with salinity. Soil pH had a non-significant relationship with initial plant height and leaf area growth, but a significant negative relationship with plant height growth. Plant height growth was significant positively affiliated with soil water content. Initial plant height, initial leaf area and leaf area growth showed a non-significant relation to soil water content (Table 1). I fitted linear mixed models explaining soil organic matter with vegetation removal as one of the explanatory variables. This gave two models: the model with lowest AIC showed that organic matter had significant positive relations with soil pH and soil water and relations with vegetation (Constant = 9.644 (33.49), Soil pH = -7.97 (2.38)***, Soil water content = 1.440 (0.32)***, AIC = 120.7), but in the second best model, which should be considered because it had an Δ AIC of 0.7 compared to the best model, removal of vegetation had a non-significant negative effect with pH and organic matter as the most important predictors (Constant = -3.31 (34.47), Removal of vegetation = -1.54 (1.38), Soil pH = -6.89 (2.42)***, Soil water content = 1.51 (0.33)***, AIC = 121.4).

Table 1. The reproduction, plant size and growth in *Herminium monorchis*. (A) Number of fruits set, adjusted for number of flowers. (B) Size of developed fruits. (C) The probability for a plant to keep its inflorescence until fruit maturity. (D) Plant height at flowering stage. (E) Growth in height from flowering stage to fruit maturity stage. (F) Plant total leaf area at flowering stage. (G) Growth in leaf area from flowering stage to fruit maturity.

| | <i>Dependent variable:</i> | | | | | | |
|--------------------------------|----------------------------|----------------------|---------------------------|-------------------------|-----------------------|-------------------------|-------------------------|
| | Number of fruits | Fruit size | Set fruit (0/1) | Initial plant height | Plant height growth | Initial leaf area | Leaf area growth |
| | <i>generalized linear</i> | <i>linear</i> | <i>generalized linear</i> | <i>linear</i> | <i>linear</i> | <i>linear</i> | <i>linear</i> |
| | <i>mixed-effects</i> | <i>mixed-effects</i> | <i>mixed-effects</i> | <i>mixed-effects</i> | <i>mixed-effects</i> | <i>mixed-effects</i> | <i>mixed-effects</i> |
| | (A) | (B) | (C) | (D) | (E) | (F) | (G) |
| Vegetation removal | -1.599*** (0.318) | 4.896 (9.045) | - | NA | 4.197** (2.065) | NA | -16.648 (212.094) |
| Initial plant size | -2.860*** (0.835) | - | - | NA | NA | NA | NA |
| Plant height growth | 6.226*** (0.699) | 16.508*** (5.307) | - | NA | NA | NA | NA |
| Leaf area growth | -0.422 (0.284) | -4.371** (1.785) | - | NA | NA | NA | NA |
| Veg. removal:Height growth | - | -13.333 (9.367) | - | NA | NA | NA | NA |
| Veg. removal:Leaf growth | - | 8.423*** (2.700) | - | NA | NA | NA | NA |
| Init. plant size:Height growth | 2.300** (0.751) | - | - | NA | NA | NA | NA |
| Number of fruits | NA | NA | NA | NA | 0.841*** (0.088) | NA | 10.253 (8.942) |
| Soil organic matter | -0.084* (0.033) | - | -0.112* (0.059) | - | - | -13.656 (15.576) | 36.166 (28.779) |
| Practical salinity | 0.002*** (0.0005) | - | 0.002** (0.001) | - | - | 0.124 (0.185) | - |
| pH | - | - | - | 333.240 (293.185) | -19.769*** (4.693) | -120.866 (236.519) | 589.699 (370.376) |
| Soil water content | - | - | - | 31.650 (24.175) | 1.686** (0.813) | 53.144 (32.585) | -25.459 (65.767) |
| pH:Soil water | - | - | - | -4.053 (3.507) | - | - | - |
| Constant | -2.649 (1.908) | -6.280 (5.232) | 6.379 (4.587) | -2480.373 (2019.173) | -22.679 (77.897) | -1703.103 (2473.665) | -4017.206 (4710.977) |
| Observations | 56 | 21 | 56 | 56 | 21 | 56 | 21 |
| Log Likelihood | -73.8672 | -33.219 | -32.979 | -264.352 | -65.019 | -403.959 | -124.352 |
| Akaike Inf. Crit. | 169.700 | 82.437 | 73.958 | 540.704 | 144.039 | 821.917 | 264.703 |
| Bayesian Inf. Crit. | 192.013 | 90.794 | 82.059 | 552.856 | 151.350 | 836.095 | 273.059 |

Note:

*p<0.1; **p<0.05; ***p<0.01

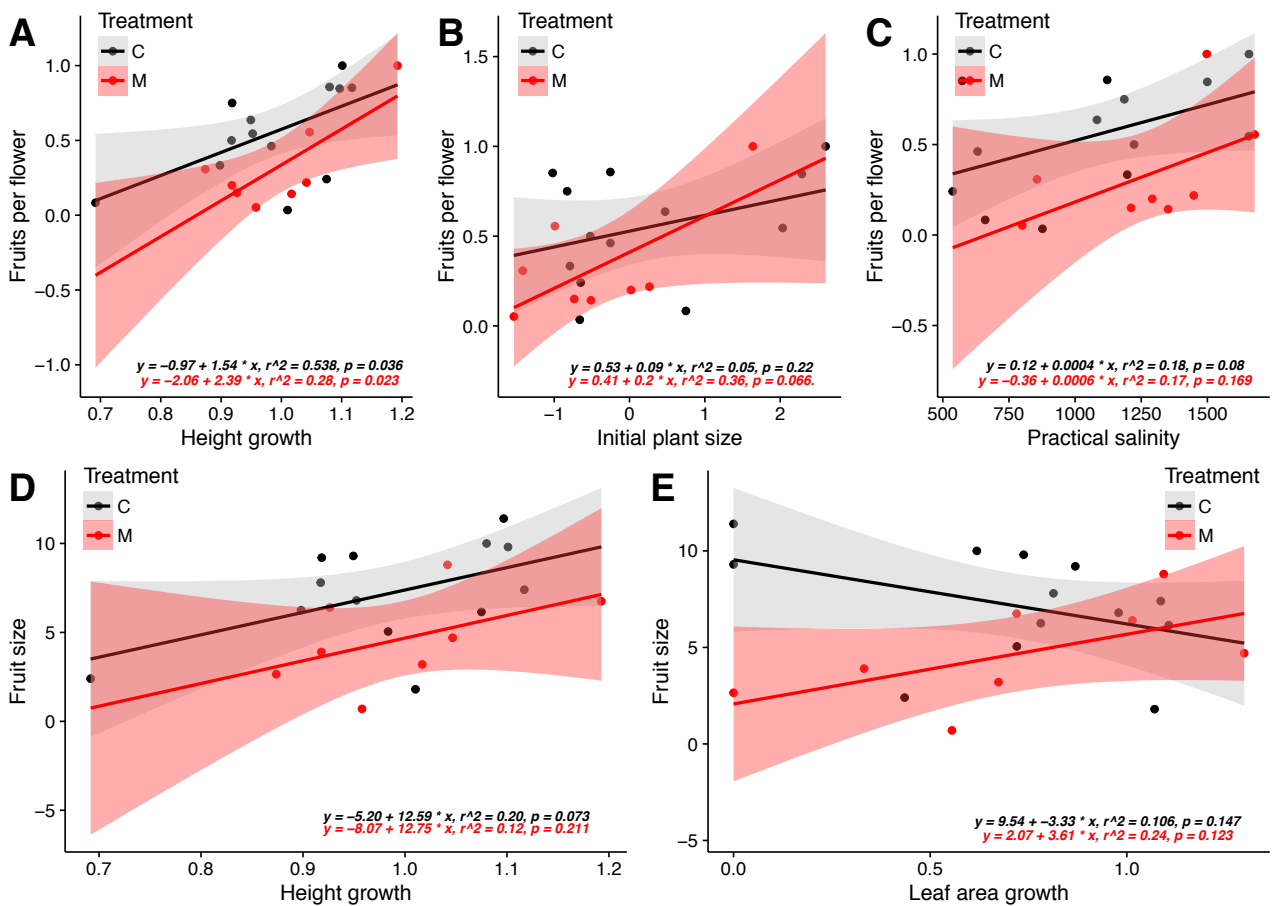


Figure 3. The reproduction of *Herminium monorchis* under experimental grazing (Treatment M) and under control (Treatment C) conditions. Removal of vegetation appears to have had a negative effect on plant reproduction and growth. (A) Fruits per flower and plant height growth. (B) Fruits per flower and initial general plant size (principal component). (C) Fruits per flower and soil practical salinity in microsiemens. (D) Fruit size and plant height growth. (E) Fruit size and leaf area growth. This plot shows a interaction between vegetation removal and leaf area growth, where removal of vegetation decreases leaf area growth with a subsequent negative effect on fruit size.

4. DISCUSSION

The results suggest that the decrease of *H. monorchis* populations in Norway is caused by changes in agricultural land use and the number of goats. The simulated grazing experiments showed that removal of vegetation around the orchids allegedly had a positive effect on fruit size and plant height growth, but a negative effect on the number of fruits produced per flower and leaf area growth. There was no significant relations were found between conspecific plant density and plant reproduction, size or growth, but the number of fruits set was positively related to soil salinity.

4.1 HISTORICAL DATA

The decrease in the total area of mowed natural meadows and the amount of goats in Norway since 1907 appears to have had a negative impact on the amount of *H. monorchis* populations. It is possible that the general change in agriculture, or some other associated unmeasured factor have caused the decrease in mown natural meadow area, number of goats and number of *H. monorchis*

populations, and that the relationship between them is purely coincidental. To examine this further, I fitted two models with natural mown area and year, respectively, as explanatory variables for the number of orchid populations. Natural mown area was the best explanatory variable, suggesting that it may have had a causal effect on the reduction in the number of *H. monorchis* populations in Norway since 1907. This is not surprising, since *H. monorchis* is perennial and allegedly a weak competitor, which makes it unlikely to thrive on tilled and fertilized areas.

Goats are browsers and feed on shoots and leaves of higher growing, and often woody, vegetation, and this suppression of woody plant species could be the reason for the positive relationship with *H. monorchis*. Sheep and cattle, on the other hand, are grazers, and feed on lower growing vegetation like grasses and forbs, which could have a direct negative impact on *H. monorchis* through loss of inflorescence and/or leaves to herbivory. The number of sheep and cattle have not decreased (SSB, 1907-1999), and had no relationship to the number of *H. monorchis* populations. A possible relationship between *H. monorchis* populations and sheep and cattle could have been masked by an increasing practice of keeping animals in fenced cultivated areas or in housings.

4.2. REMOVAL OF VEGETATION

I found that experimental grazing through removal of vegetation had a negative effect on the number of fruits set per flower. Removal of vegetation also had a significant positive effect on plant height growth, and plant height growth had a positive relationship to the number of fruits per flower (Figure 3A). In 2014 and 2015, Kravdal et al. (2016) monitored the three Norwegian *H. monorchis* populations and their different population processes. In this study they compared *H. monorchis* individuals occurring in an area that previously had been grazed by cattle (although enclosed in a fence and excluded from grazing since 2014), to individuals growing outside the fence, exposed to cattle grazing. Here they found that in 2014 and 2015, a significant higher proportion of the individuals growing outside the fence set became fertile inflorescence compared to the individuals inside the fence. In 2014, the *H. monorchis* individuals that occurred outside the fence had more biomass compared to those that occurred inside. Nevertheless, they found no difference in *H. monorchis* biomass in 2014, or individual density in both 2014 and 2015, between the grazed and the fenced individuals. This could imply a positive relationship between cattle grazing and the survival and reproduction of *H. monorchis* in general, although my findings suggests that grazing could have a negative effect on reproduction during the fertile stage of this plant.

It could be expected that a more plants (in terms of biomass) on the soil surface could facilitate more water from deeper soil layers to the top soil layer through belowground plant vascular tissue, but there was no difference in soil water content between control quadrates and quadrates where vegetation was removed. All orchids are heterotrophic and nourished by mycorrhizal fungi (Leake, 1994). Thus, it could be possible that reducing aboveground biomass reduced the flux of carbon from the plant layer to the belowground mycorrhiza networks, causing a negative impact on mycobial activity in the soil, which in turn limited resource flux from mycorrhizal fungi to the orchids. The mycorrhizal fungi connected to *H. monorchis* is not known, and orchid mycorrhiza taxa are highly host specific (Leake, 1994). Hence, orchid mycorrhiza does not form symbiotic interactions with other plant groups, and should not be directly affected by changes in the plant community unrelated to the host orchids. Moreover, there was no significant difference in organic matter content between manipulated and control quadrates, although soil organic matter had a non-

significant relationship with removal of vegetation content in the second best model. But removal should have had a stronger effect on organic matter for us to conclude that there was a difference in biomass flux to the soil. Hence it is unlikely that the reduction in the orchids reproduction by vegetation removal is caused by related changes in water facilitation, carbon flux and mycorrhizal relationships

In this study removal of vegetation had a significant negative effect on the amount of fruits set and leaf area development. Fruit size appears to have been affected by vegetation removal in a positive interaction with leaf area growth, where the vegetation removal decreased leaf area growth, which had a positive relation to fruit size. In control plants, on the other hand, there appears to have been a negative relationship between fruit number and leaf area growth (Table 1B, Figure 3E). Orchids have been shown to have reduced photosynthetic capacity and growth under too high solar radiation (He et al., 2004), and plant responses to ambient UV-doses are often shown in a bell-shaped curve, where elevated levels of UV increases plant height and the amount of fruits to a point where the UV level becomes too high, causing plant height and fruit amount to rapidly drop (Brodführer, 1955, Qaderi et al., 2008). Moreover, *H. monorchis* populations have been shown to have negative relations to higher temperatures, with fewer fertile individuals emerging after years with warmer summer temperatures than average (Wells et al., 1998). Leaf temperature increases substantially under sunlight exposure (Leigh et al., 2006), which means that the *H. monorchis* individuals that had surrounding vegetation removed could have suffered from temperature increase through a higher exposure of direct sunlight. It is possible that plants that invested more into fruit development had fewer resources available for leaf area development, and that the plants were stressed under experimental grazing. Fruit size was reduced by vegetation removal under lower leaf area growth, but reached the same fruit size levels as the control plants under higher leaf area growth. This could mean that plants with more resources available could have invested more resources into both fruit size and leaf area development, and that these plants were less affected by the experimental grazing.

In this study, experimental grazing was conducted on plants that had already been established. Removal of vegetation through grazing creates gaps, which allow more photosynthetically active radiation to reach the lower vertical levels of the vegetation (Mitchley and Willems, 1995), and this has been shown to allow more seeds the opportunity to germinate and subsequently increase population density (McConnaughay and Bazzaz, 1987, Jacquemyn et al., 2011). Thus, it is possible that the negative effects of vegetation removal on the reproduction of *H. monorchis* could be mitigated by a higher rate of seed germination and a subsequent higher recruitment the following season. Moreover, in the study area, *H. monorchis* develops its leaves and inflorescences later than several graminoid and forb species. Thus, it is possible that the critical competition for light may have taken place before the reproductive stage, and that vegetation removal could have had a different effect on *H. monorchis* if it was conducted earlier in the season. Nevertheless, it has been shown that some wet meadow plant species had lower survival under vegetation removal treatment at early stages (Kelemen et al., 2015). This underlines how plant community disturbance through grazing and mowing could have different effects at different magnitudes for different plant species.

4.3 CONSPECIFIC PLANT DENSITY AND FLORAL DISPLAY

I could not find any results suggesting that the reproduction of this *H. monorchis* population is related to conspecific plant density at patch level or floral display. I suggest three explanations for this: a) Pollinator visitation was not related to plant density or floral display in this population b) different plant densities or floral display could have had different effects on pollinator visitation rates, but all the *H. monorchis* individuals received sufficient pollination nevertheless c) different pollinator visitation rates could have occurred under different plant densities and number of flowers, but the expected difference in fruit set could have been masked by plants being resource limited and unable to take advantage of excess. Moreover, I did not look at how the reproduction of this *H. monorchis* population was affected by population size and population density. Hence it is possible that by explaining reproduction in relation to patch level only, I could have overlooked possible relations happening at population level.

4.4. PLANT SIZE AND GROWTH

Number of fruit sets and fruit size had a significant positive relationship with plant height growth (Figure 3A&D). Number of fruits also explained plant height growth significantly. This could mean that plants with more resources available could invest into both number of fruits and plant height growth. It could also mean that plants with higher potential to set more fruit, perhaps from successful pollination, invested more into the development of fruits and inflorescence. Figure 3B shows that initially larger plants set more fruits per flower, and furthermore that fruit size was negatively related to leaf area growth, but positively related under vegetation removal (Table 1, Figure 3E). Most plants showed a decrease in leaf area (growth ratio <1), and this could mean that with lower amounts of resources available and/or a low potential for sexual reproduction could have allocated more resources for leaf area development and increase the photosynthetic active area to store more resources in the tubers, or that perhaps successfully pollinated plants allocated more resources to reproduction rather than leaf area development.

4.5. SOIL CONDITIONS

H. monorchis has already been shown by Økland (1996) to tolerate saline conditions. My results show that under more saline conditions the plants produced fruits per flower (Figure 3C), with a higher probability of keeping their inflorescence to fruit maturity. Little appears to be known about how salinity may affect reproduction and growth in orchids. Mold (2012) lists all known plant taxa that are known or suggested to be halophytic, and only two orchid species are mentioned (*H. monorchis* not included), suggesting that salt tolerance is uncommon or occur to an unknown extent in orchids. Plants growing in saline conditions suffer from salt stress, which generally causes reduced water uptake and growth rate. Salt may also be accumulated to toxic levels in the leaves, which cause earlier senescence of the leaves and a reduction in the plants active photosynthetic area (Munns, 2002). Practical salinity had no relation to leaf area or plant height growth, and this lack of observed senescence to salinity could imply that *H. monorchis* could be less susceptible to salt stress. *H. monorchis* is unlikely to avoid salt stress completely and gain from more saline conditions, hence it is more probable that *H. monorchis* may suffer less from salt stress than its neighbouring plant competitors, and thus face less competition where salt concentrations are higher.

The plant height growth of *H. monorchis* showed positive relationships with soil water content. This is not surprising, since the plant is known to grow in humid areas. Nevertheless, this relation could be biased the presence of *Menyanthes trifoliata*, which grows in very humid soil, even under

water, and has very large leaves that rise above neighboring plants. Hence it is possible that *H. monorchis* experienced intensified competition for light in more humid soil and grew taller to reach better light conditions. Soil water content had a positive effect on plant height growth, which in turn had a positive effect the number of fruits set. Higher water levels could have dampened the possible negative effects of salt stress on plant growth, but there was no significant interaction effect between soil water content and practical salinity on plant reproduction or growth.

The number of fruits set and probability to keep inflorescence until fruit maturity for *H. monorchis* individuals in this population showed a negative response to soil organic content. Higher levels of soil organic matter have been shown to increase the soil ratio between nitrogen and phosphorus (N:P) ratio in wetlands (Bedford et al., 1999). In the study area, higher N:P ratios could have occurred under higher soil organic contents and could have increased competition from plant species that could better take advantage of the higher N:P ratios to the *H. monorchis* individuals. Thus, organic matter could have had an indirect negative effect on reproduction through increased competition from a possible higher N:P ratio. If this is the case, it implies that *H. monorchis* could be susceptible to suffer from nitrogen deposition as well, one of the largest global change stressors on ecosystems (Vitousek et al., 1997a). I suggest this to possible relation to be examined more thoroughly in further studies of *H. monorchis* in Norway, and this could also be investigated with a correlative and experimental approach, by comparing the historical data of *H. monorchis* populations to historical levels of nitrogen deposition and to look at the general performance of *H. monorchis* under ambient nitrogen levels (for example by exposing *H. monorchis* individuals to the different ambient soil nitrogen levels predicted in different nitrogen deposition scenarios).

4.6. REPRODUCTION

The *H. monorchis* population in Skjellvik had a lower fruit set per flower (45%) than a population measured at Öland, Sweden in 1979 (70-95%) (Nilsson, 1979). The amount of fruit sets of the Skjellvik population is also lower than the average in other populations of European nectar-rewarding orchids (Neiland and Wilcock, 1998). This means that the Skjellvik population could have a relative low reproductive success in terms of fruit production. Variation in plant characters and reproductive success between populations has also been found in other orchids (Ehlers et al., 2002), and the relatively lower amount of fruits set in Skjellvik could imply that the population is occurring under non-ideal environmental conditions. Nevertheless, it has been shown that the number of fertile *H. monorchis* individuals in a population can vary greatly between years in relation to amount of rainfall and summer temperatures in previous years (Wells et al., 1998, Kravdal et al., 2016), and this could possibly apply for the number of fruits set as well. This could mean that longer time series are required to pull correct conclusions about the reproductive performance of *H. monorchis*.

The Skjellvik population had a larger mean number of flowers on each inflorescence (25.21) than the Öland population (19.34). More flowers on the inflorescence have been shown to increase pollinator visits in total per flower, pollinia removal and fruit set in orchids (Ehlers et al., 2002, Brys et al., 2008). In my results, neither fruit set, fruit/flower-ratio or fruit size can be explained by the most proximate measures of pollinator visitation I had available (conspecific inflorescence density and number of flowers per inflorescence). Moreover, I observed that the plants had a large variation in fruit size (ranging from 1.6 mm² to 11.4 mm². When an orchid is visited by a pollen-

carrying pollinator, the pollinia is deposited as a whole and may provide sufficient amounts of pollen after only one visit, which is possibly why pollen load normally has no effect on seed size and amount in orchids (Petit et al., 2009). This could rule out pollen limitation as an explanation for the observed large differences in fruit size, and indicates that some other factor must have had an impact on seed production in this population, suggesting that the reproduction of this population could be more strongly constrained by other environmental conditions than pollination.

4.7. CONCLUSIONS AND MANAGEMENT IMPLICATIONS

My findings suggest that vegetation removal through livestock grazing may not necessarily have a positive effect on plant reproduction, and subsequently that livestock grazing should be used with care if to be used as a management measure on populations of endangered plant species. Taking the general positive effects of cattle grazing found by Kravdal et al. (2016) in to consideration, a possible management measure for the current extant *H. monorchis* populations could be to apply cattle grazing early in the growth season, but to enclose the plants in a fence and exclude cattle grazing during the fertile stage. But considering the results from the historical data, I believe that the use of non-selective vegetation removal, through mowing, or establishing grazing regimes with browser livestock like goats, could prove more valuable for the conservation of *H. monorchis* than the heavy vegetation removal applied by grazing cattle. The results from the historical data probably imply that there is little viable habitat left in Norway for *H. monorchis* to establish on. Additionally, the mycorrhizal fungus species connected to *H. monorchis* is unlikely to be present in all habitats otherwise suitable for *H. monorchis* establishment, which will make germination of seeds and establishment of plants highly unlikely. Thus, the best approach for the conservation of this species could be to increase population size in already extant populations, where we already know the habitat to be suitable. Browsers, like goats, can be used to remove competitive vegetation to create better conditions for plant establishment, before sowing out *H. monorchis* seeds or transplanting viable plants. For establishing new populations, reintroduction could be attempted in humid meadows, pastures and fens where the soil can remain undisturbed from tillage and fertilization.

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