**Inland Norway University**

Faculty of Applied Ecology, Agricultural Sciences and Biotechnology

Nadine Closset

# Master thesis

# Brainworm (*Elaphostrongylus rangiferi)* abundance in wild reindeer (*Rangifer tarandus tarandus*) in relation to gastropod densities

Master in Applied Ecology

2021

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# Abstract

*Elaphostrongylus rangiferi* is a nematode parasite in reindeer *(Rangifer tarandus)* which can cause considerable neurological damage and could affect the survival chances of the last European wild tundra reindeer. The parasite has terrestrial gastropods as intermediate hosts. Previous research has shown that the development of *E. rangiferi* inside gastropods is highly temperature-dependent, with faster development at higher temperatures. Additionally, the prevalence and abundance of *E. rangiferi* has previously been reported to be lower in reindeer grazing at high altitudes, but whether this difference in infection rate is connected to gastropod densities is unknown.

Here I showed that overall prevalence and abundance of *E. rangiferi* was significantly higher for reindeer that are in summer pastures with a high predicted gastropod density. These areas were mainly forested areas at low altitudes. The prevalence and abundance of *E. rangiferi* changed over time, with maximal output in faecal samples during early spring. Overall prevalence and abundance were considered to be relatively low compared to other studies on both wild and semi-domesticated reindeer.

Previous studies suggested that the higher prevalence of *E. rangiferi* in reindeer grazing in low altitudes was mainly connected to higher temperatures. My results provide a new dimension into understanding risk areas for *E. rangiferi* transmission. My study showed that the parasite was common in the wild reindeer population of Rondane, a population from which there was little prior information. In light of climate change, prevalence and density of this parasite in reindeer is expected to increase. This makes *E. rangiferi* a parasite of increasing concern. My findings, in combination with previous research, could be used by both reindeer herders and conservation managers for management and mitigation strategies of reindeer to prevent future outbreaks of *E. rangiferi*.

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**The contribution of NC to this thesis was as follows:**

This thesis work was performed as part of a bigger project (Klimasyk rein) which aims to build predictive models for the prevalence and abundance of *Elaphostrongylus rangiferi* in both semi-domesticated and wild reindeer in Trøndelag county, Norway. The study design for faecal sampling was in collaboration with project partners and supervisors: Nadine Closset, Mattanja Stuut, Andrea Miller and Alina Evans (Inland Norway University of Applied Sciences), Geir Rune Rauset (NINA), Torill Mørk and Rebecca Davidson (Norwegian Veterinary Institute) and Hannah Vineer (University of Liverpool). Study design for gastropod sampling was done by NC in collaboration with project partner MS. NC determined study areas and plot locations. Fieldwork for both gastropods and faeces collection was performed by NC, with support from AE and MS. GR provided GPS data for the collared reindeer. Laboratory work was designed and supervised by RD but performed by NC. HV provided advice regarding the landscape use and abundance models, but NC was responsible for all data handling, statistical analysis, interpretations and writing this master thesis.

# 2.  Introduction

Wild reindeer (*Rangifer tarandus*) numbers are declining on a global scale due to increasing anthropogenic pressure and climate change. In the last century, 70% of undisturbed habitat from the last European wild tundra reindeer (*Rangifer tarandus tarandus*) has been lost to anthropogenic pressure (Nellemann et al., 2003). These wild reindeer now consist of 26 fragmented sub-populations in Norway which are managed in 24 management areas in Southern Norway (Figure 1) (Andersen & Hustad, 2004; Nellemann et al., 2001). The size of the herds vary from a few hundred to more than 10.000 reindeer (Bevanger & Jordhøy, 2004).



## Wild reindeer areas in Norway

National wild reindeer areas
Other reindeer areas
European wild reindeer regions
Reindeer husbandry areas

1  Setesdal Ryfylke
2  Setesdal Austhei
3  Skaulen Etnefjell
4  Våmur - Roan
5  Brattefjell - Vindeggen
6  Blefjell
7  Hardangervidda
8  Norefjell - Reinsjøfjell
9  Oksenhalvøya
10  Fjellheimen
11  Nordfjella
12  Lærdal - Årdal
13  Vest - Jotunheimen
14  Sunnfjord
15  Førdefjella
16  Svartebotnen
17  Reinheimen - Breheimen
18  Snøhetta
19  Rondane
20  Sølnkletten
21  Tolga Østfjell
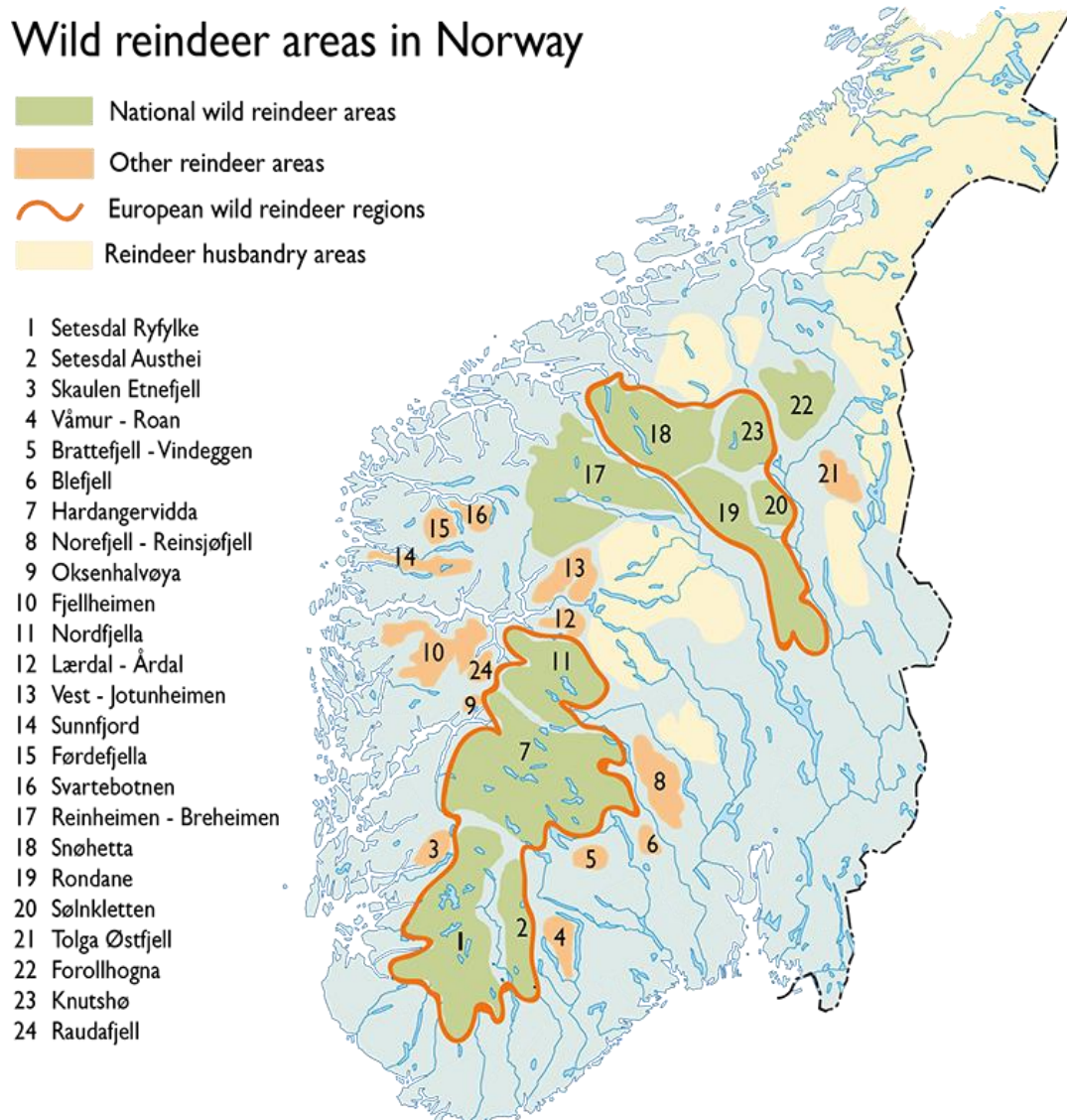22  Forollhogna
23  Knutshø
24  Raudafjell

Figure 1: Current wild reindeer areas in Norway (Andersen & Hustad, 2004).
Numbers represent the 24 management areas.

In northern latitudes, the climate is getting warmer and wetter (IPCC, 2013; Hanssen-Bauer et al., 2003). These changes in climate pose a threat to reindeer in different ways: warmer winters are correlated with lower body weights in calves, which has a negative effect on survivability (Weladji & Holand, 2003). Climate change also causes a change in vegetation structure and composition (IPCC, 2013). Changes in vegetation structure may limit the food availability for reindeer (O'Brien, Sygna, & Haugen, 2004). Warm periods in the winter can result in an icy crust on the deep snow that prevent reindeer from reaching the vegetation, often referred to as locked pastures (Ottersen et al., 2001). Another important effect of climate change, yet often overlooked, is the increase of frequency, distribution and intensity of certain parasitic diseases (Okulewicz, 2017). One such parasite that is expected to be of increasing concern is *Elaphostrongylus rangiferi,* better known as 'brainworm' (Davidson et al., 2020).

*Elaphostrongylus rangiferi* is a nematode parasite in reindeer which has terrestrial gastropods (snails and slugs) as intermediate hosts. It has been reported to be transmittable to other cervid and non-cervid species (Handeland, Skorping, & Slettbakk, 1993; Sten, Blackmore, & Skorping, 1997). Although previous research shows that the parasite can cause severe damage in sheep (*Ovis aries*) and goats (*Capra aegagrus*), the parasite does not seem to complete its lifecycle in these hosts as it doesn't reach the skeletal muscle. As a result, larvae (L1) are not found in these faeces. However, the possibility of transmission to sheep is of concern considering that these animals often share grazing areas with reindeer (Davidson et al., 2020; Handeland & Skorping, 1992; Handeland, Skorping, & Slettbakk, 1993). Other cervid species, such as red deer (*Cervus elaphus*) and moose (*Alces alces*), are also susceptible to *E. rangiferi*. In these animals the lifecycle can be completed, although the parasite doesn't produce as many larvae as in reindeer and the clinical effects are less intense (Handeland et al., 2019; Sten, Blackmore, & Skorping, 1997).

The severity of neurological symptoms caused by *E. rangiferi* is dosage-dependent (Handeland, 1994). However, most infected reindeer will show no visual symptoms. Any symptoms present become particularly visible when the animals are experiencing thermal stress (extreme weather), or are pushed to exercise (Bakken & Sparboe, 1971, 1973). The stance of the animal is characterized with an arched back, lowered pelvic region and a general weakness (Bakken & Sparboe, 1973; Handeland et al., 1994). Poor or reduced growth has also been recorded in calves (Handeland & Norberg, 1992). This deterioration of the physical condition can affect the survival chances of the reindeer, especially during winter (Handeland et al., 1994). Most studies focussing on *E. rangiferi* in reindeer have been conducted on semi-

domesticated reindeer. Very little is known about *E. rangiferi* infections in wild reindeer in Norway (Handeland et al., 2019).

As illustrated in Figure 2, reindeer become infected once they eat gastropods which contain brainworm larvae at the third moulting stage (L3) (Miskevich, 1964). Once the infected gastropod is ingested by reindeer, the parasite travels via the circulatory system to the central nervous system in reindeer. Once there, they mature to adults within 48 to 90 days. The parasite can be found in the spinal cord and the subdural (brain) spaces of the central nervous system (Handeland, 1994). After 90 days and up until 182 days after ingestion, more adults are found on skeletal muscle tissue. The adults produce eggs which are transported via the circulatory system to the lungs, where they hatch. The larvae (L1) are then coughed up, swallowed and passed in the faeces where they can survive for a long period of time (13 months) under extreme cold temperatures up to -80°C (Kummeneje, 1973; Lorentzen & Halvorsen, 1976). Upon infection with these L1 by gastropods, the larvae develop from the first to the third moulting stage (L3) and becomes infectious again to reindeer.
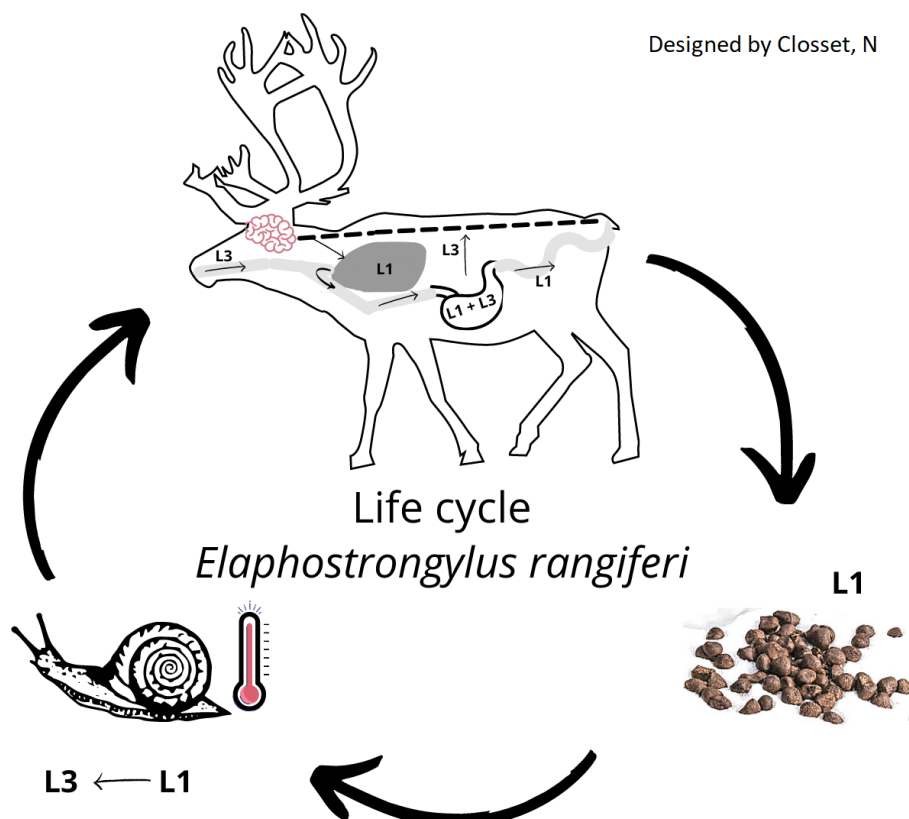


*Figure 2: Lifecycle of Elaphostrongylus rangiferi. L1 = larvae stage one, L3 = larvae stage three.*

The speed of development from L1 to L3 in gastropods is dependent on the timing of infection and the annual temperatures in the weeks post-infection. Development from L1 to L3 only occurs above eight degrees Celsius (°C), and the speed of development increases with higher temperatures (Schjetlein & Skorping, 1995; Skorping, 1982). With an average summer temperature under eight to ten °C, most L1 won't be able to develop to L3 prior to snow coverage and would thus have to overwinter in gastropods (Schjetlein & Skorping, 1995). However, with high temperatures (mean above 15°C) this development could take less than two weeks (Halvorsen & Skorping, 1982; Skorping, 1982). Correspondingly, outbreaks in reindeer appear to be more common after summers with high temperatures. When the conditions are favourable, the two-year lifecycle of the parasite could change into a one-year lifecycle (Vineer et al., 2021). This makes *E. rangiferi* of increasing concern given the climatic predictions for Norway (Davidson et al., 2020; IPCC, 2013; Halvorsen, 2012; Handeland & Slettbakk, 1994).

However, the rate of this development is dependent on the species of gastropod. On average, the larvae need 249 degree-days to develop from L1 to L3 in the gastropod (Vineer et al., 2021). Degree-days are defined as the temperature of the observation minus the temperature threshold (8°C) multiplied by the accumulated days post-infection when L3 are first observed in gastropod hosts (Kutz, Hoberg, & Polley, 2002). There are several gastropods living across Fennoscandia which have proven be suitable hosts for *E. rangiferi* such as *Arion silvatucus, Deroceras leave, Discus ruderatus, Euconulus fulvus* and *Trichia hispida* (Skorping & Halvorsen, 1980). Development from L1 to L3 in these hosts is rapid. It is unclear how the densities of these species are distributed across Fennoscandia. General gastropod density is highly related to the soil moisture level (Godan, 1983). Other environmental factors might also determine the specific habitat selection such as pH, shelter from solar radiation, food availability and temperature. Gastropod preference usually tends to shady, moist and cool areas with a high level of calcium (Boag, 1985; Andersen & Halvorsen, 1984; Walden, 1981). There is also a knowledge gap of whether differences in gastropod densities are connected to infection prevalence (i.e. number of infected reindeer) and abundance (i.e. number of parasites) of *E. rangiferi* (Davidson et al., 2020; Handeland et al., 2019).

Usage of summer pasture is crucial for the abundance of *E. rangiferi*, because this is when reindeer mostly get infected (Vineer et al., 2021; Handeland et al., 2019; Halvorsen, 2012; Handeland & Slettbakk, 1994; Halvorsen et al., 1980). A study from Handeland et al., (2019) suggested that infection prevalence might be lower in animals grazing at higher altitudes,

because of the relative lower temperature. Although there was no clear pattern in the relation between altitude and *E. rangiferi* prevalence, significantly lower prevalence of *E. rangiferi* were found in the two reindeer populations grazing at higher altitudes compared to the four other populations at lower altitudes.

My hypothesis is that brainworm prevalence and abundance in reindeer can be explained by the gastropod density in their summer grazing area. Gastropods are mainly found in wet, calcium rich vegetation below the tree line. Areas with higher altitude are generally dryer, colder and have sparser vegetation compared to areas with lower altitudes. These areas are thus less likely to have many gastropods. Prevalence and abundance of *E. rangiferi* is expected to be lower in reindeer which graze in areas with lower gastropod densities. There is currently very limited information about *E. rangiferi* infections in wild Norwegian reindeer (Handeland et al., 2019) and the distribution of gastropods in these reindeer grazing areas. As my part of a larger project (Klimasyk rein, see below) and to test my overall hypothesis, I will do the following: 1) create a predictive model of gastropod density and 2) compare infection prevalence and abundance between wild reindeer using different summer pastures.

Note: This study is part of a bigger project (Klimasyk rein) which aims to build predictive models for the prevalence and abundance of *E. rangiferi* in both semi-domesticated and wild reindeer in Trøndelag county, Norway. It aims to gain a better understanding of outbreaks of *E. rangiferi* in relation to the landscape use of the herd in combination with local climate data. Both Mattanja Stuut, who is doing a Master thesis on the related topic on semi-domesticated reindeer (Stuut, 2021), and I focus on landscape use of reindeer in relation to *E. rangiferi* prevalence and abundance.

# 3. Materials and methods

## 3.1 Study area

Rondane is a mountainous area located in south-central Norway. Topography of the area ranges from 441 to 2176 meters above sea level (MASL) and is characterised by its alpine-boreal vegetation, which mainly exists of boulder fields, lichen heath and dwarf shrub heath at high altitudes. At lower altitudes the main vegetations are pine and birch forests with lichen and heath (Aune-Lundberg & Bryn, 2018).

The study followed five wild reindeer fitted with a GPS collar containing temperature sensors (Vectronics Vertex Plus with Iridium satellite communication) in and around Rondane in the heart of Inland county in Norway. The living area of these individuals corresponded with number 19 in Figure 1. The five collared reindeer (individually called 247, 248, 249, 258 and 6455) moved together in roughly two groups. Individuals 248, 249 and 258 moved together in winter in a large group of approximately 1800 reindeer. The other individuals, 247 and 6455, moved together in a group of approximately 350 reindeer. Collaring and handling of the animals was performed by the Norwegian Institute for Nature Research (NINA) under ethical approval (Norwegian Food Safety Authority FOTS ID 15116) and regional permits (Norwegian Environment Agency).

NINA – The Norwegian Institute for Nature Research, provided shape files containing information about the vegetation types and reindeer positions. The reindeer positions were provided as a point layer and gave the positions of the collared reindeer every three hours starting in March 2019 and ending in May 2020. The vegetation data was provided as an AR50 raster layer (Heggem, Mathisen, & Frydenlund, 2019) and was categorized based on land cover vegetation with a resolution of 25x25m. Elevation data was a N50 raster with a resolution of 5x5m (Norwegian Mapping Authority, 2017). All data was downloaded and imported into QGIS (QGIS Development Team, 2020).

## 3.2 Data Collection

The data collection in the field consisted of three parts. The first part focused on the difference in area use between the two reindeer groups, and the second part on the gastropod density in different areas. The last part consisted of faecal sampling of the two reindeer groups with subsequent analysis for *E. rangiferi* prevalence and abundance. Prevalence of *E. rangiferi* was defined as the number of infected individuals (reindeer). Abundance of *E. rangiferi* was defined as the number of L1 parasites inside the faeces (Bush et al., 1997; Margolis et al., 1982).

### 3.2.1 Area use

The point data of the reindeer positions from March 2019 to May 2020 was filtered to only include the summer months of 2019 (June, July and August) as it was the most likely infection period for reindeer (Handeland et al., 2019; Halvorsen et al., 1980). I constructed 95% Minimum Convex Polygons (MCP; Worton, 1987) with R version 4.0.3 (R Core Team, 2020) in RStudio (RStudio Team, 2018), using the *adehabitatHR* package (Calenge, 2006). I used these MCP's with the purpose of identifying the general summer grazing area for each individual. 95% MCP's were preferred over 100% MCP's in order to exclude places that the reindeer rarely visit and thus limits the risk of overestimating the summer grazing area (Burt, 1943; Hayne, 1949). Individual summer home ranges (Figure 3) revealed that some individuals had overlapping summer gazing areas. Ultimately, the reindeer were divided into roughly two groups (North = 247 and 6455, South = 248, 249 and 258). The need to separate them into two groups, rather than looking at them individually, was because it was not possible to differentiate individuals while collecting faecal samples in the field. In winter, the reindeer naturally moved in these two groups which made this division evident. Individuals in the same group had similar vegetation use in the summer.
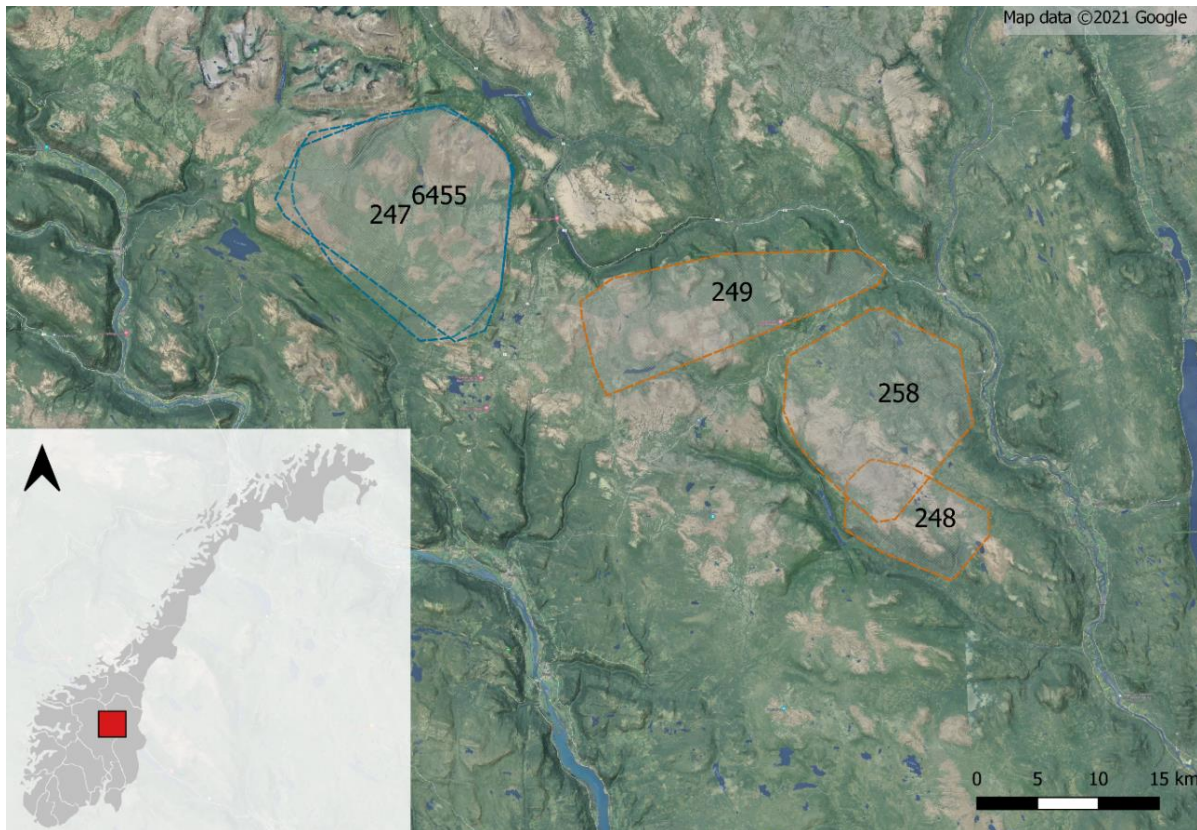
*Figure 3: Home range for the five collared reindeer. Each polygon with dotted line represents the 95% MCP for each reindeer for the months of June-August 2019. Polygons with a blue dotted line belong to the northern group. Polygons with an orange dotted line belong to the southern group. Numbers in these polygons are the names of the individual reindeer. Brown areas indicate mountainous vegetation types and the green areas are the forests in lower altitudes. Blue is water.*

## 3.2.2 Gastropod density and diversity

Based on methods described in Andersen and Halvorsen (1984), I estimated the density of gastropods by qualitative visual searching plots. The selection of which vegetation types were to be sampled was based on the main habitat use of the reindeer during the summer (see Appendix 1) and habitat within their summer grazing area which were likely to be favoured by gastropods (Andersen & Halvorsen, 1984). The location of the sampling squares within the selected habitat was done beforehand by randomly assigning GPS points, taking into account site accessibility. Within these representative vegetation types, two to three sampling squares were placed per location with a dimension of one square meter ($m^2$). Distance between the sampling squares was always more than ten meters. There were a total of 49 sampling squares, all within – or close to – the summer grazing pastures of the reindeer. All of the sampling was done at the end of August/beginning of September in 2020 (weeks 35-37).

The squares were visually searched for gastropods for ten minutes by two observers. This included moving rocks and vegetation. The number of gastropods found in each sampling square was noted and the gastropods found were placed in labelled plastic bags with plot identifier numbers. The collected gastropods were determined to species level using morphological keys (White-McLean, 2011). When unsure, species were determined to genus level or labelled as 'Unidentified'. Additional comments were noted about the dominant vegetation of each sampling square. Other variables, like pH and humidity, were also measured in addition to the number of gastropods per sampling square. A simple meter (Vistefly 3-in-1 soil tester) was used to measure the pH and humidity level in the soil. Spatial interpolation was later done in QGIS to determine the elevation, slope and aspect of each sampling square.

### 3.2.3 *Elaphostrongylus rangiferi* prevalence and abundance over time

*Fieldwork*

Faecal pellets were collected to analyse the prevalence and abundance of *E. rangiferi* within each reindeer group. This collection was done over the course of eight months, from early spring to mid-autumn. The sampling was carried out at least once for each reindeer group in March/April, June and October 2020 to see how the infection pressure changed over the seasons. In March, more pellets were collected from the southern reindeer group because of lack of sunlight during the day when the northern reindeer group was too far in the mountains. In August, only the southern reindeer group was sampled. It was not possible to sample the northern group because of the start of the reindeer hunting season.

Recent GPS positions from the reindeer were visited (within 48 hours) and faeces were collected from the ground in the area around these positions. The order of the sampling of the reindeer groups was dependent on their proximity to the road or hiking trails. Each pile of pellets was assumed to be one individual. The pellets were stored in separate labelled plastic bags and reindeer group, coordinates and date were recorded. The sampling was opportunistic, but the aim was to collect 18-20 samples at each sampling event, with each sample containing at least five to ten grams of faeces. The samples were frozen at -18°C until further analysis could be carried out. Freezing the samples was possible because of the ability of brainworm to survive in extreme freezing temperatures (-80°C) for over a year (Lorentzen & Halvorsen, 1976).

## *Faecal analysis*

A modified version of the Baermann technique (Baermann, 1917) was used to estimate the prevalence of L1 *E. rangiferi* larvae in the reindeer faeces. Because of the simplicity of the technique, alternative materials can be selected to isolate the larvae. In this case, conical plastic glasses were used instead of funnels (Cheng et al., 2018; Graeff-Teixeira et al., 1997; Gajadbahar et al., 1994;).

The faeces (five to ten grams) were placed in a medical gauze (10 x 10cm, eight layers) which was folded into two layers. The precise weight of the faeces used was recorded. The medical gauze was sealed at the top, so it became a parcel, and was secured with a stick. The parcel was then submerged in a conical glass filled with lukewarm water with the stick resting on the edges of the glass, so the parcel was completely suspended in the water. Over time, the *E. rangiferi* larvae inside the faecal parcel came out and sunk to the bottom of the glass. The Baermann sample was always left overnight at room temperature between 18-20°C to give the larvae have enough time to come out.

The following day, the bottom five millilitres (ml) left was transferred to a labelled test tube. The glass was rinsed, and this liquid was also added to the test tube. The test tube was then centrifuged at 1700g for three minutes. The supernatant in the test tube was removed with a single use Pasteur pipette to one ml. The final one ml was mixed to homogenise the sediment at the bottom. A subsample of 100µl was placed on a glass microscope slide and checked for the presence of L1 under a compound microscope using a 10x objective lens. The larvae were morphologically identified and counted. When no larvae were present in the first sub-sample, a second 100µl subsample was taken from the sediment for counting. The abundance, larvae per gram (LPG) was calculated for each Baermann sample using the following formula:

$$LPG = \frac{\{\Sigma\ larvae\ \times\ V\ water\ in\ tube\ (1000\mu l)\ /\ V\ water\ examined\ (100\mu l)\}}{weight\ faeces\ (g)}$$

## 3.3 Statistical analyses

All statistical analyses were performed in R version 4.0.3 (R Core Team, 2020) using in RStudio (RStudio Team, 2018). The threshold for statistical significance was set at $p < 0.05$. The fit of all subsequent models were explored using the *DHARMa* package (Hartig, 2020). *DHARMa* was also used to detect possible over/under dispersion, autocorrelation and outliers on the best-fitted models.

### 3.3.1 Area use

*Table 1: Original classified vegetation types as defined in vegetation map AR50 (Heggem, Mathisen, & Frydenlund, 2019) and allocated vegetation types for this study.*

| Original vegetation type | Allocated vegetation type | Original vegetation type | Allocated vegetation type |
|---|---|---|---|
| Forest, high production | | Forested bog | Forested bog |
| Forest, intermediate production | Forest | Open bog | Open bog |
| Forest, low production | | Agriculture | |
| Forest, unproductive | | Fenced pastures | |
| Forest, unregistered | | Infrastructure | |
| Open area, vigorous vegetation | | Bare rock and boulder fields | *NA* |
| Open area, intermediate vegetation | Open area | Snow/ice | |
| Open area, sparse vegetation | | Freshwater | |

The spatial points of the reindeer from June to August 2019 were interpolated with the vegetation and altitude raster so that each recorded reindeer position had a specific vegetation type and altitude. The original vegetation types consisted of 16 different categories. For the ease of making models, the original categories were allocated into only four categories: forest, open area, forested bog and open bog (Table 1). All other vegetation types were omitted due to the low GPS point counts in these places (*NA*). The differences in vegetation use, altitude and air temperature between the two reindeer groups were explored and tested with a Wilcoxon test (Wilcoxon, 1945). Results were presented with lower and upper quartiles (Q1 and Q3).

### 3.3.2  Gastropod density and diversity

*Data exploration and model building*

A model was made to estimate gastropod densities. The response variable was the number of gastropods (snails and slugs) found per plot. Several explanatory variables were collected in the field (vegetation, humidity, pH, GPS position, time), whilst distance to treeline and aspect were calculated using the point sampling tool in QGIS (Table 2). I considered all non-forested areas above 800 meters above sea level (MASL) to be above the treeline. The distances to the treeline from sampling positions above the treeline were defined as positive values, whereas the distance to the treeline from a position below the treeline were defined as negative values.

The protocol from Zuur, Ieno, & Elphick (2010) was used as a guideline for data exploration. Heavily correlated variables (>0.7; Dormann et al., 2013) were removed before building models. Relations between X and Y variables were explored and checked for potential outliers. All continuous variables were scaled before building models. I used the *glmmTMB* package to build Generalized Linear Mixed Models (GLMM) for its robustness and ability to model over dispersed distributions to make predictive gastropod density models (Brooks et al., 2017).

*Table 2: Explanatory variables considered for modelling gastropod density estimations with data type and description.*

| Name explanatory variable | Type | Description |
|---|---|---|
| Distance to treeline | Numeric | Distance to treeline; All values below the treeline were defined as negative, all values above were defined as positive. This combines both information about elevation and presence above or below the treeline. |
| Vegetation | Factor | Vegetation type where the plot was sampled: forest, open area, forested bog or open bog |
| Humidity | Numeric | Humidity level of the topsoil. |
| pH | Numeric | pH level of the topsoil. |
| Aspect | Factor | Direction that the slope faces: North, East, South and West. |
| Group | Factor | In which reindeer grazing area the plot was located: northern or southern reindeer group. |
| Time | Numeric (in time format 'hms') | Starting time of the visual search of the sampling square. |

## *Model selection*

A total of 13 models were built including a null and full model. The models were compared using Akaike's information criterion with a correction for the small sample size (AICc). The best-fitted models were those with the lowest AICc score. Models were considered to be equally good when their ΔAICc were less than two (Al Halwachi, Yakovlev, & Boek, 2004; Sugiura, 1978; Akaike, 1973). The fit of the models was tested to detect flaws in the five models with the lowest AICc score.

## *Predictive forecasting*

Using the estimates from the best-fitted model, I created a predictive raster using the *raster* package (Hijmans, 2020). Missing values were filled up with GDAL's 'fill nodata' function (Open Source Geospatial Foundation, 2021). GPS locations of the reindeer from months June to August 2019 were interpolated to explore the difference in gastropod density between the two reindeer groups in the summer using a Wilcoxon test (Wilcoxon, 1945).

### 3.3.3 *Elaphostrongylus rangiferi* prevalence and abundance over time

*Data exploration and model selection*

First, a model comparing *E. rangiferi* abundance between the two reindeer groups was built. Similar to the gastropod density data, the brainworm abundance was also count-data with excess zeros. Therefore, I also fitted a negative binomial GLMM with the *glmmTMB* package (Brooks et al., 2017). The difference of *E. rangiferi* abundance between the two reindeer groups was tested by making a model with *E. rangiferi* abundance as a response variable, reindeer group as a fixed explanatory variable and month of when the faeces were collected as a random explanatory variable. This way the difference of *E. rangiferi* abundance between the two reindeer groups was tested while taking the timing of the faecal collection into account.

The difference of *E. rangiferi* abundance over time was also explored by building a negative binomial GLMM. This time, the month was a fixed explanatory variable while reindeer group was a random explanatory variable. This was used to look at how the abundance of L1 changes over the seasons while accounting for the differences in the two reindeer groups. Post-hoc analyses were applied on the model investigating difference in abundance of *E. rangiferi* over time to see how each month differed from each other. This was done using the *multicomp* package, which runs multiple comparisons in models with a Tukey test (Hothorn, Brentz, & Westfall, 2008).

Lastly, a model was built comparing *E. rangferi* prevalence between the two reindeer groups. The structure of this model was similar to the abundance model by having reindeer groups as a fixed explanatory factor and by taking sampling month into account as a random explanatory factor. However, this time a GLMM with a binomial distribution was used, with prevalence (presence/absence) of *E. rangferi* as the response variable.

# 4. Results

## 4.1 Area use

Reindeer groups differed significantly in their vegetation use during the summer months ($p <$ 0.001, Wilcoxon test). The GPS positions revealed that the northernmost reindeer group were more in mountainous vegetations (open areas) during the summer, whereas the southern group was mostly in the forest. MCPs of the animals during the summer were used as a reference for the summer grazing area (Figure 4). The southern reindeer group seemed to be more in open areas outside of these summer months (Appendix 1).
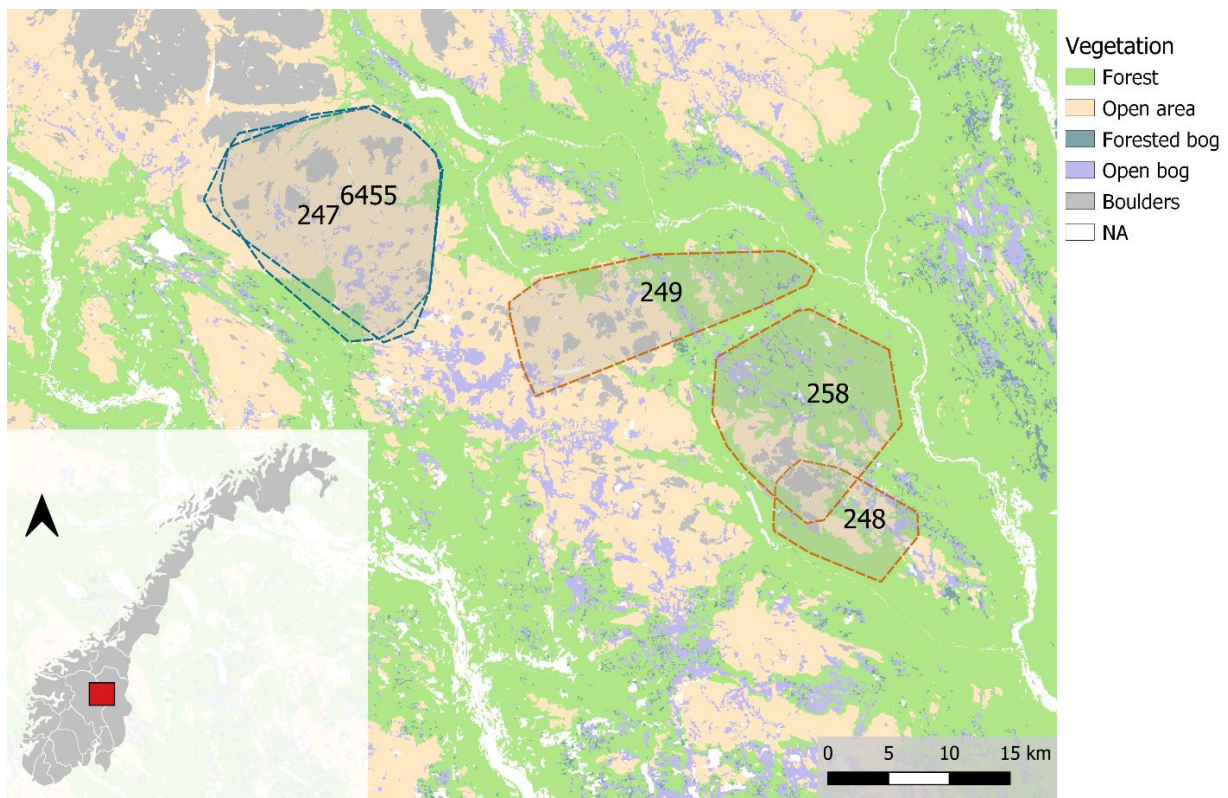


*Figure 4: Vegetation in the summer grazing areas of the individual reindeer. Each polygon with dotted line represents the 95% MCP for each reindeer for the months of June-August 2019. Polygons with a blue dotted line belong to the northern group. Polygons with an orange dotted line belong to the southern group. Numbers in these polygons are the names of the individual reindeer.*

The northern reindeer group was mostly in the mountains, with an average altitude of 1200m (Q1 = 1163, Q3 = 1297) whereas the southern group was more below the tree line with an average altitude of 849m (Q1 = 689, Q3 =1028) (Figure 5). Altitude in summer differed significantly ($p < 0.001$, Wilcoxon test) between the two reindeer groups. Similarly, the air temperatures recorded by the GPS collars of the northern group (average of 17,24°C, Q1 = 13°C, Q2 = 20°C) were significantly lower ($p < 0.001$, Wilcoxon test) than the southern group (average of 18,63°C, Q1 = 15°C, Q3 = 22°C).
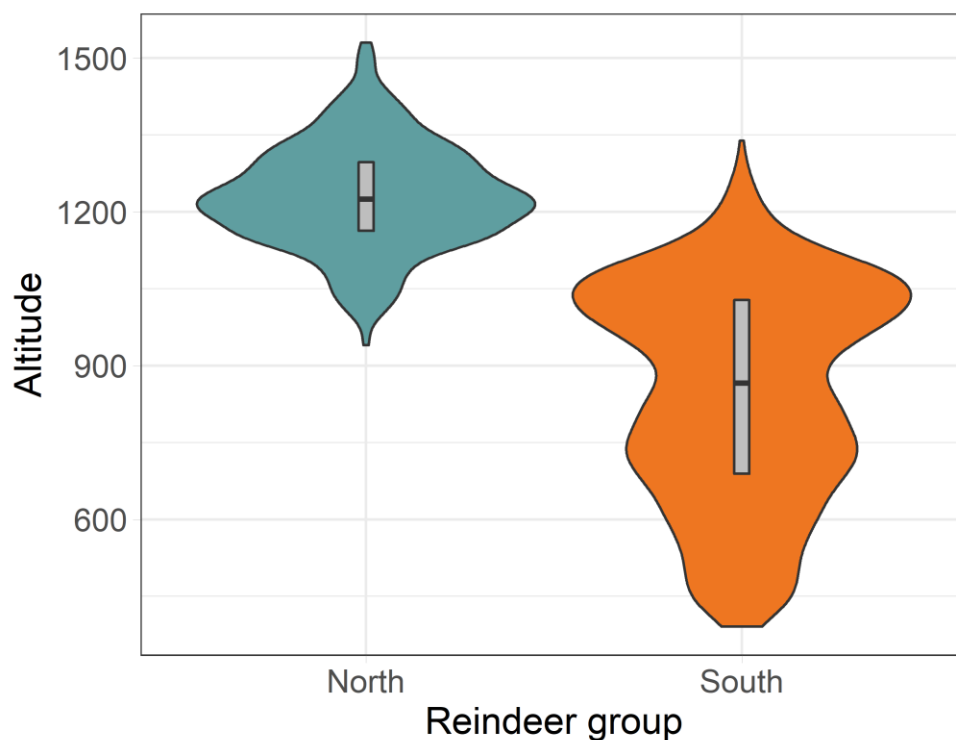


*Figure 5: Violin plot showing the distribution of altitude (in MASL) for the recorded positions during summer (June – August 2019) for the two reindeer groups (n=3515 GPS locations) with a boxplot (grey box).*

## 4.2 Gastropod density and diversity

A total of 103 terrestrial gastropods were collected across the 49 sample plots. None of the sample plots above the treeline had any gastropods. Overall, six different species were found. The different species found, and the habitat they were found in are listed in Appendix 2. There were 13 gastropods which remained unidentified.

### *Model selection*

An overview of the five best-fitted models and their AICc value are shown in Table 3. M1 and M2 were alike because their ΔAICc were <2. M1 was ultimately selected because it had the lowest AICc score and a good fit. The best-fitted model was afterwards tested both with and without the random factor 'Group' and/or 'Time'. When fitted with the random factor, the residuals showed a clear pattern whereas when fitted without the random factor the model showed a relatively good fit. I therefore chose to leave these random factors out of the model. Post-hoc tests revealed that the model proved to be robust enough as almost no significant results were found in the residual diagnostics ($p > 0.05$). One significant result was found when exploring the spatial autocorrelation ($p = 0.009$, Moran I test).

*Table 3: Five models to estimate the gastropod density with the lowest AICc.*

| Name model | Model | AICc value | ΔAICc |
|---|---|---|---|
| M1 | Gastropods ~ Distance to treeline * Vegetation | 171.02 | 0.00 |
| M2 | Gastropods ~ Vegetation + Aspect + pH + Humidity | 172.55 | 1.53 |
| Mfull | Gastropods ~ Vegetation + Aspect + Distance to treeline + pH + Humidity | 177.19 | 6.17 |
| M3 | Gastropods ~ Vegetation + Distance to treeline + pH | 177.60 | 6.58 |
| M4 | Gastropods ~ Vegetation + Distance to treeline + Humidity | 180.06 | 9.04 |

## Model interpretation

The gastropod density significantly decreased with an increase of the distance to treeline in the 'open area' vegetation type ($p$ <0.001). This means that mountainous areas generally had very low gastropod abundance. This corresponded with observations from the field, where none of the sample plots above the treeline had gastropods. In contrast, forest vegetation well below the treeline had a significant positive effect on the gastropod abundance (p <0.001).

## Predictive forecasting

The predicted gastropod densities inside the summer grazing area are presented in Figure 6. The figure illustrates the estimated gastropod density per m$^2$, where higher densities are darker red in colour.
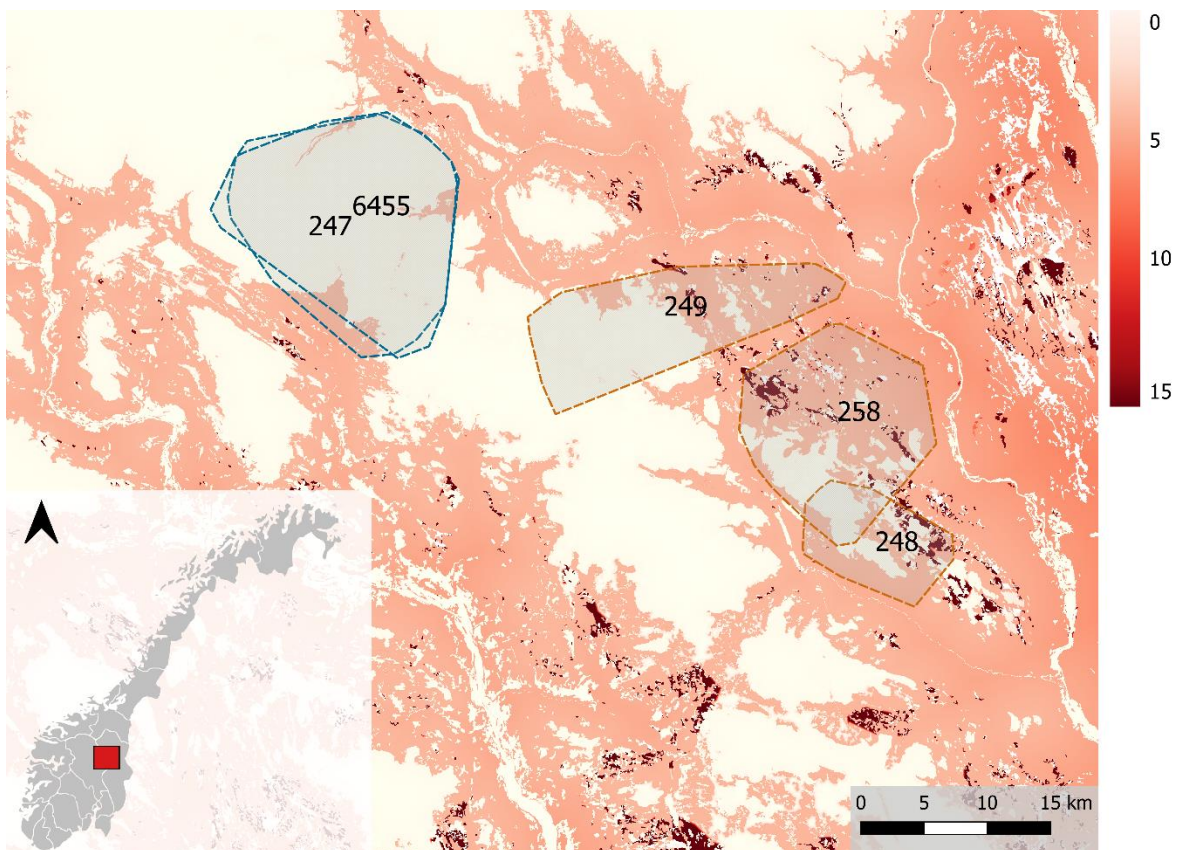


*Figure 6: Estimated gastropod density per m$^2$ in and around the summer grazing area of the reindeer. Each polygon with dotted line represents the 95% MCP for each reindeer for the months of June-August 2019. Polygons with a blue dotted line belong to the northern group. Polygons with an orange dotted line belong to the southern group. Numbers in these polygons are the names of the individual reindeer. Shades of red represent the estimated gastropod density per m$^2$ with darker red indicating higher gastropod densities.*

Interpolated GPS points revealed that the southern reindeer group had a significant higher gastropod density on their visited locations as compared to the northern group (Figure 7, $p < 0.001$, Wilcoxon test). Most of the estimated gastropod densities per $m^2$ for the northern reindeer group were close to zero, whereas the densities for the southern group could be grouped in three different density estimates, between zero and one, around three and six or more gastropods per $m^2$.
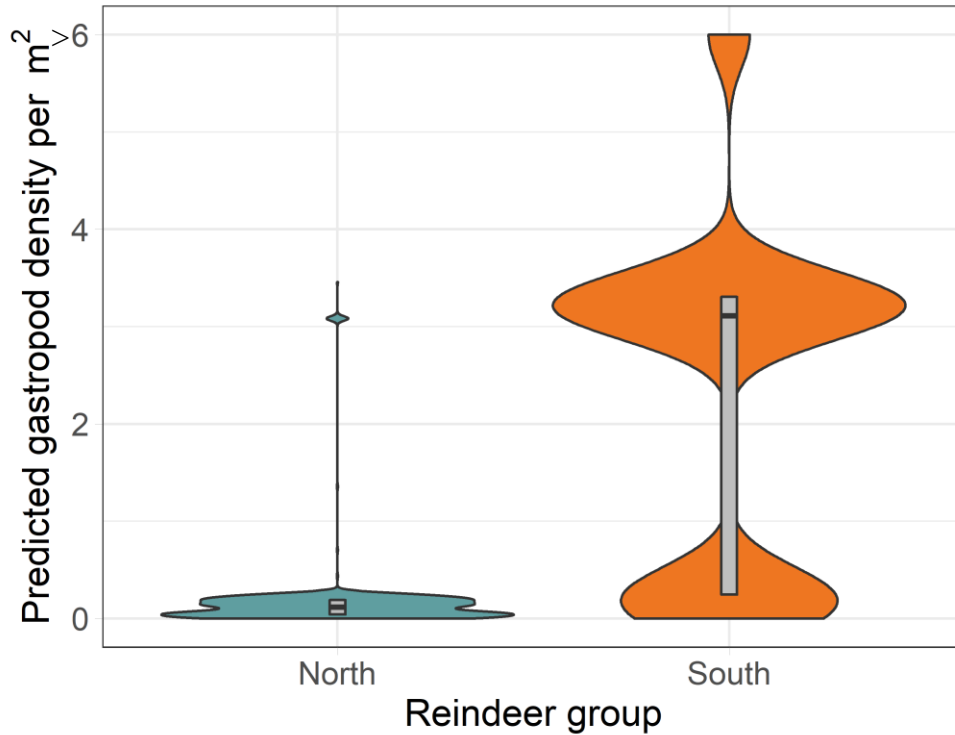


*Figure 7: Violin plot showing estimated gastropod densities per m² for the recorded positions for individual reindeer during summer (June – August 2019) for the two reindeer groups (n=3515 GPS locations) with a boxplot (grey box).*

## 4.3 *Elaphostrongylus rangiferi* prevalence and abundance over time

In total, 194 faecal samples were analysed to study the change in *E. rangiferi* abundance in the faeces over the seasons (Table 4). Two other models focussed on the difference between the northern ($n = 78$) and the southern reindeer group ($n = 116$). One model focussed on prevalence, while the other focussed on abundance. Model fit was explored on all best-fitted models showed no significant results which means the models had a relative good fit.

*Elaphostrongylus rangiferi* abundance decreased over time, with the highest abundance (LPG) in March (Figure 8). March was also the month where most faecal data was collected ($n = 73$). Post-hoc analysis revealed that March had significantly higher *E. rangiferi* abundance compared to August ($p = 0.001$) and October ($p < 0.001$). However, there was no significant difference in abundance between March and June ($p = 0.06$). June had significantly higher abundance compared to October ($p < 0.001$), but not to August ($p = 0.27$). Abundance differed significantly between August and October ($p = 0.01$). The southern reindeer group had a significantly higher *E. rangiferi* abundance compared to the northern group ($p = 0.003$). Prevalence between the two reindeer groups was also significantly different, with the southern group having a higher overall *E. rangiferi* prevalence ($p = 0.02$).

*Table 4: Elaphostrongylus rangiferi abundance as found in the faeces. Parasite abundance (median, mean, min and max) were calculated where the parasite was present.*

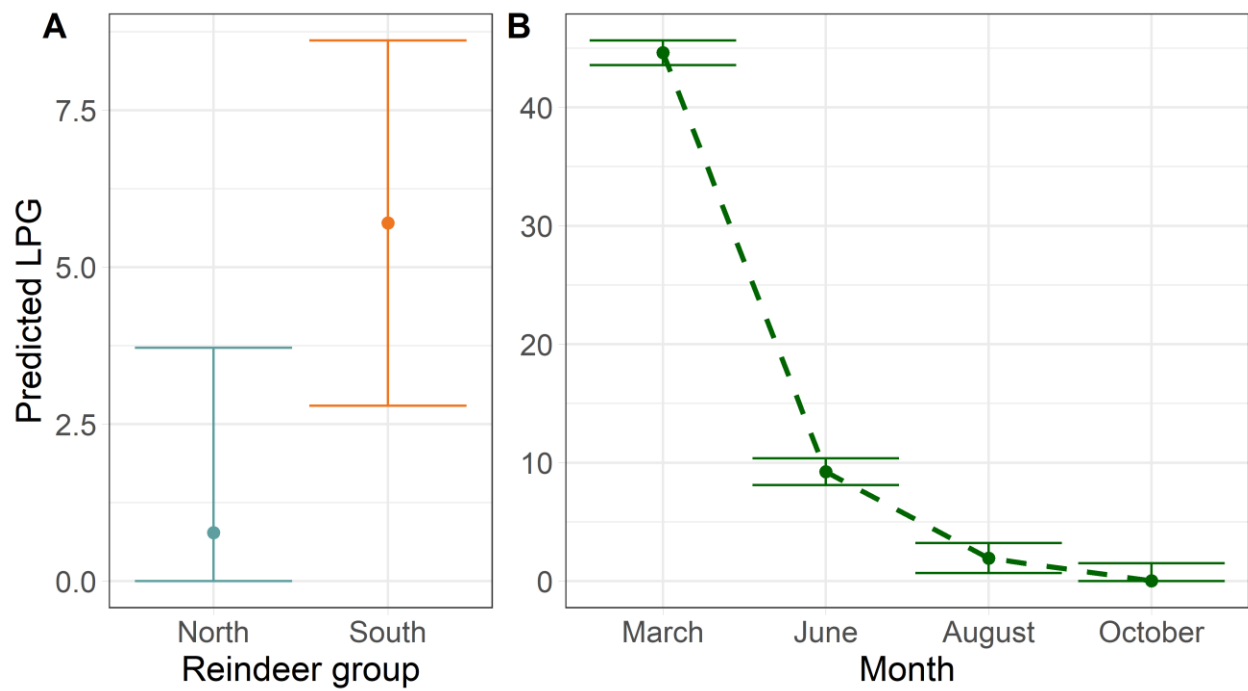| Reindeer group | Timing | n total | n positive | Prevalence | Abundance (LPG) | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | median | mean | min | max |
| North | March | 17 | 3 | 18% | 136.7 | 122.2 | 30 | 200 |
| North | June | 21 | 4 | 19% | 1 | 0 | 1 | 6 |
| North | October | 20 | 1 | 5% | 1 | 1 | 1 | 1 |
| South | March | 56 | 30 | 54% | 65 | 151.4 | 2 | 1129 |
| South | June | 20 | 6 | 30% | 80 | 109.8 | 7 | 247 |
| South | August | 20 | 10 | 50% | 4.6 | 7.3 | 1 | 16 |
| South | October | 20 | 0 | 0% | 0 | 0 | 0 | 0 |

*Figure 8: (A) Predicted mean E. rangiferi abundance (LPG) for each reindeer group with standard deviations. (B) Predicted mean E. rangiferi abundance (LPG) over time with standard deviations.*

# 5. Discussion

This study followed five wild reindeer fitted with GPS collars which were roughly divided into two groups based on summer grazing areas. I validated my hypothesis that abundance and prevalence of *E. rangiferi* was lower in reindeer that were grazing in areas with lower estimated gastropod densities.

## 5.1 Gastropod density and diversity

Gastropod density was predicted to be highest in forested areas well below the treeline. The higher densities in forests can possibly be explained by the ecological demands of gastropods. Gastropods usually prefer calcium-rich habitats such as calcium rich fens or birch forests (Andersen & Halvorsen, 1984). Indeed, gastropods were not found above the treeline in this study, and I had predicted the gastropod density above the treeline to be extremely low. These findings are consistent with the study from Andersen and Halvorsen (1984), which also rarely found snails above the treeline. In contrast to this study, no snails were found close to calcium-rich bogs above the treeline. This is possibly due to the low sample-size in such bogs. Why the treeline was so important is not clear but could be explained by unfavourable climatic conditions. Preferred habitat of gastropods are mainly moist areas with high pH where they are sheltered from the natural elements such as solar radiation and temperatures above 21°C ( Andersen & Halvorsen, 1984; Godan, 1983, Walden; 1981). Gastropods are more exposed to the natural elements above the treeline. On sunny days, gastropods often seek shelter, as they generally tend to avoid temperatures above 21°C (Dainton, 1989; Boag, 1985). Below the treeline there are also more nutrients coming from dead plant material which are directly available to the gastropods (Andersen & Halvorsen, 1984).

While the gastropod density model was consistent with findings in the field, it did not take the weather condition of the sampling date into account, as gastropods seek shelter on sunny days. However, collection was always done on cloudy/rainy days, so this should not have significantly influenced the counts. Visual detection of the gastropods within the sampling squares was sometimes a challenge because of the small size of most gastropods, with some being even less than two millimetres. Bigger gastropods would naturally be easier to detect than smaller ones. This means that it is possible that not all gastropods were detected within

the sampling timeframe and the predicted densities were likely underestimated (Boulinier et al., 1998).

It was only possible to make simple models because of the small sample size (n = 49). Having too many interactions would have caused over parameterization in the model (Shapiro, 1983). However, the best-fitted model proved to be robust given that the fitted vs. predicted residuals showed no significant results, except for spatial autocorrelation. This spatial autocorrelation means that sample plots which are close to each other have similar ecological properties (Legendre, 1993). This would apply in this case, as the plots were mostly selected dependent on where the reindeer grazed in the summer. For the northern group, the sample plots were primarily at higher altitudes: above, or just below the treeline. The gastropod density here was generally low, whilst the southern group was mainly in the forest at lower altitudes, where gastropod densities were higher.

A previous study from Skorping & Halvorsen (1980) investigated gastropod species which could possibly act as intermediate hosts by experimentally infecting gastropods with *E. rangiferi*. Out of the six gastropod species identified in this study, there was one, *Discus ruderatus*, which proved to be a better suited host compared to the other gastropod species that were found in this study. Experimental infection showed that the growth inside this snail species is rapid and has a high level of infection when fed on faecal pellets with L1 larvae. Interestingly, this species was found in four plots in forested areas, whereas only in one plot in open areas in this study. The one found in open area was a plot close to a small stream. The most abundant gastropod species in the study, *Nesovitrea spp.,* was only found in forested areas (forest and forested bog). Development of *E. rangiferi* inside this gastropod is considerably delayed with small numbers of parasite development. The same is true for *Cochlieopa lubrica*, which was mainly found in open areas, and *Arion subfuscus*, which was mainly found in the forested habitats (forest and forested bog), with only two observations in open areas. Skorping and Halvorsen's experimental study (1980) found a particularly low degree of infection in *Punctum pygmaeum*. The brainworm larvae were not able to develop in this snail species. No information is available on brainworm larval development in *Tandonia spp*, the last identified species. There were a total of 13 (out of 103) gastropods which remained unidentified.

## 5.2 Area use and *Elaphostrongylus rangiferi* abundance in reindeer

The northern reindeer group studied was, in the summer months, mostly found in open areas in the mountains while the southern group mostly used the forest at lower altitudes. Why there was this habitat difference between these two reindeer groups is not clear. Reindeer generally prefer open areas in the mountains during the snow-free months, and usually avoid forests at lower elevations because of higher temperatures and possible insect harassment (Skarin et al., 2004, 2010). Other important factors influencing reindeer movement include weather parameters, human activity and forage quality of the vegetation (Skarin et al., 2010), which might have differed between the areas from the two studied reindeer groups and could thus explain why the southern reindeer group was mainly found in the forest at lower altitudes. The study was mostly focused on the area use of the reindeer in the summer months because the development to the infective L3 stage in the gastropods is highly temperature dependent. This is also why it was assumed that reindeer mostly got infected during these months (Davidson et al., 2020; Vineer et al., 2021).

Interestingly, the temperatures as recorded by the GPS collars were significantly higher in the southern reindeer group. Areas at lower altitudes are generally warmer, which might also explain why the *E. rangiferi* abundance was significantly higher in the southern group. Higher temperatures imply that the lifecycle inside the snail is shorter (Davidson et al., 2020; Halvorsen, 2012; Vineer et al., 2021). However, the difference in temperatures between these two reindeer groups was not investigated further in this part of the project. The recorded temperature by the collars might have also been influenced by other factors such as body temperature of the reindeer, solar radiation etc. Nevertheless, temperatures recorded by GPS collars on animals have been proven to be representable of ambient air temperatures (Ericsson et al., 2015). Effects of temperature and gastropod species distribution on *E. rangiferi* prevalence and abundance in reindeer are currently being further investigated as another part of the Klimasyk rein project.

One of the greatest limitations in this study was that it was not possible to model environmental variables together with *E. rangiferi* abundances, because it was not possible to know exactly when and where the reindeer became infected. Nonetheless, this study found a significant difference in *E. rangiferi* abundance between the two reindeer groups, which was correlated with a significant difference in area use between the two groups during the preceding summer.

This implies that area use in summer had a noteworthy effect on the *E. rangiferi* abundance. Previous literature has found a relationship between the *E. rangiferi* abundance and altitudes where reindeer grazing at lower altitudes during the summer months had higher *E. rangiferi* abundance (Halvorsen, 2012; Handeland et al., 2019). These studies mostly explained the difference in *E. rangiferi* abundance with colder temperatures at higher altitudes. The outcomes of this study, however, also pointed out that the difference in *E. rangiferi* abundance in various altitudes could plausibly be associated to the gastropod density in these areas and provides a new dimension into understanding risk areas for *E. rangiferi* transmission.

Compared to other studies on semi-domesticated and wild reindeer (Halvorsen, Skorping, & Hansen, 1985; Handeland et al., 2019), overall *E. rangiferi* abundance and prevalence in wild reindeer in this study was considered to be relatively low, with an exception for August in the southern reindeer group. Prevalence here was relatively high. However, this exception could be because of the low sample size. Clinical signs caused by *E. rangiferi* are dosage-dependent (Handeland, 1994) and were thus presumably minimal for these wild reindeer. Nonetheless, even minor clinical signs could be critical for wild reindeer, especially in winter. Most studies on *E. rangiferi* have been performed on semi-domesticated reindeer and very few on wild reindeer (Handeland et al., 2019). This study provides evidence that *E. rangiferi* infections are fairly common in wild reindeer populations of Rondane and gives valuable information about the general health risk for these wild populations.

## 5.3  Seasonal output of *Elaphostrongylus rangiferi* larvae

Halvorsen, Skorping and Hansen (1985) showed that the output of L1 *E. rangiferi* larvae in reindeer had a seasonal cycle which was dependent on the sex of the reindeer. They related this to the degree of stress and the immune response in the infected reindeer. Larval outputs were highest at the times of the year when the animals were in their poorest condition or experienced most environmental stress. Females are in their poorest condition right after winter (Hyvärinen et al., 1977), which was presumably why they had their largest larval output in early spring. Larval outputs for males was highest in autumn, likely due to high environmental stress related to the rut (Halvorsen, Skorping, & Hansen, 1985). In my study, *E. rangiferi* abundance decreased as spring turned to summer and autumn. The highest *E. rangiferi* abundances were found in March 2020 and gradually declined until October, when

the sampling stopped. The natural female dominated composition of wild reindeer herds (Skogland, 1989) likely explains why my findings follow the larval output trend of females.

## 5.4 Future research recommendations

The specific impact of temperature in combination with gastropod species distribution and density on *E. rangiferi* abundance should be further investigated. A possible next step could be to calibrate the temperature data from the GPS collars with local weather stations. This would make moving reindeer samplers of their own environment (Ericsson et al., 2015). A grid map could be made with differences in thermal suitability, similar to the ones made by Vineer et al. (2021). This could be further developed to a small scale level, in combination with gastropod species distribution and density. However, it should be taken into account that gastropods seek shelter with temperatures above 21°C (Boag, 1985; Dainton, 1989). More environmental variables could also be included when modelling *E. rangiferi* abundances in addition to sampling more reindeer sub-populations. This was not possible in this study because I only studied two reindeer groups. However, if the scope of the study would be expanded to include more groups it could be achievable to do this. Similar studies to these are now running on semi-domesticated reindeer (Stuut, 2021), so future research about this would be feasible.

There also is not much information about the species distribution of gastropods in Norway (Andersen & Halvorsen, 1984). Species distribution in combination with gastropod susceptibility to *E. rangiferi* would be important components in the above-mentioned risk maps. Distribution of highly susceptible gastropod species (such as *Discus ruderatus*) are especially important in understanding the transmission risk to reindeer (Skorping & Halvorsen, 1980). This knowledge would be useful for mapping the risk of infection for each reindeer, which could then be used by both reindeer herders and conservation managers for management and mitigation strategies of reindeer to prevent future outbreaks of *E. rangiferi*.

## 5.5 Prospects of *Elaphostrongylus rangiferi* and climate change

Climate change has serious consequences on the richness and abundance of gastropods in Fennoscandia. The treeline is increasing in altitude across Fennoscandia. (Grace, Berninger, & Nagy, 2002; Smith et al., 2009). For example, in Sweden the treeline is expected to increase 223-667m in altitude in the coming 100 years, depending on the location and climate scenario (Moen et al., 2004). This means that suitable gastropod habitat is also expected to increase, with subsequent increase in gastropod density. Mountainous regions in Norway will have longer growth seasons for vegetation, which means that the periods when gastropods are active will be prolonged and therefore the time that reindeer can get infected will also be longer (Michelsen et al., 2011; Vanneste et al., 2017). In addition, gastropod species richness in Norway is expected to increase significantly in the years to come due to species distribution changes as a result of climate change (Hof, 2011). The exact composition of these species is difficult to predict. However, new species also means that there could be more possible hosts for *E. rangiferi*. An example of a new introduced species is *Arion vulgaris*. This invasive slug is considered a pest in Fennoscandia and has a higher natural nematode load and prevalence of endoparasites compared to some native gastropods of the same genus (Antzée-Hyllseth et al., 2020).

Not only the richness and density of gastropods is expected to increase, but also the speed of development of the parasite inside these gastropods. Development from L1 to the infective L3 stage is rapid when subjected to the right climatic conditions (Halvorsen & Skorping, 1982; Schjetlein & Skorping, 1995; Skorping & Halvorsen, 1980). As temperatures in Norway are rising, the normal two-year cycle of the parasite could change into a one-year cycle (Vineer et al., 2021; Davidson et al., 2020; Halvorsen, 2012).

With all factors combined, it is clear that *E. rangiferi* is a parasite of increasing concern. It is likely that the number of *E. rangiferi* outbreaks will increase in the future. This could pose a threat to the last European wild tundra reindeer as well as the livelihood of reindeer herders. Future research is needed to estimate the where and when there will be outbreaks and how management can be adapted accordingly. Reindeer herders could use alternate grazing areas, whereas wildlife managers could have more visual surveillance for reindeer that have been grazing in high-risk areas. Hunting strategies and regulations could be adapted to target those reindeer who experience physical symptoms.

# Acknowledgements

First and foremost, I would like to thank the Klimasyk rein project, funded by the Regional Research Fund Trøndelag, and everyone involved in that project. A special emphasis for Rebecca, Andrea and Alina, my supervisors, for making this master thesis possible. My thanks to Geir-Rune who was able to give me updated GPS positions of the reindeer and the fast replies on the e-mails.

Thank you to Torill, Ingebjørg and Karin from the Norwegian Veterinary Institute in Tromsø for being so welcoming and teaching me the lab-protocols. I learned a lot on this short trip and enjoyed my time above the Arctic circle. Thank you Rebecca for taking Mattanja and me in your house during this time.

For the fieldwork I thank Mattanja. She was helping me most of the times. Together we conquered many kilometres in the mountains. Rondane was sometimes unforgiving. It wasn't the easiest place to learn skiing, and in the summer it was hard to escape the mosquitos. Thankfully, we both stayed positive and would laugh about it afterwards. Nonetheless, we enjoyed the magnificent landscapes, the tranquillity of the mountains and our possibility to escape our desks.

I thank my family for their unconditional support. Even though they were sometimes confused about what I was doing exactly, they supported me all the way through. Thank you for the surprise packages with food from home to feed your 'hungry student' and for staying in touch through the many video-calls.

I would also like to thank my previous and present housemates: Petter, Anna, Jérémy and Emma. Covid-19 didn't always make it easy for our projects, but we made it. I want to thank you all for your words of support, the evenings around the fire and our nice dinners together. You made the house feel like a home.

Lastly, I would also like to thank the fellow students sitting in the 'master room' on campus Evenstad. Our daily coffee breaks and lunches together helped me too keep my head cool. I'm happy that we could work together in the same space and help each other out where needed.

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# Appendix 1
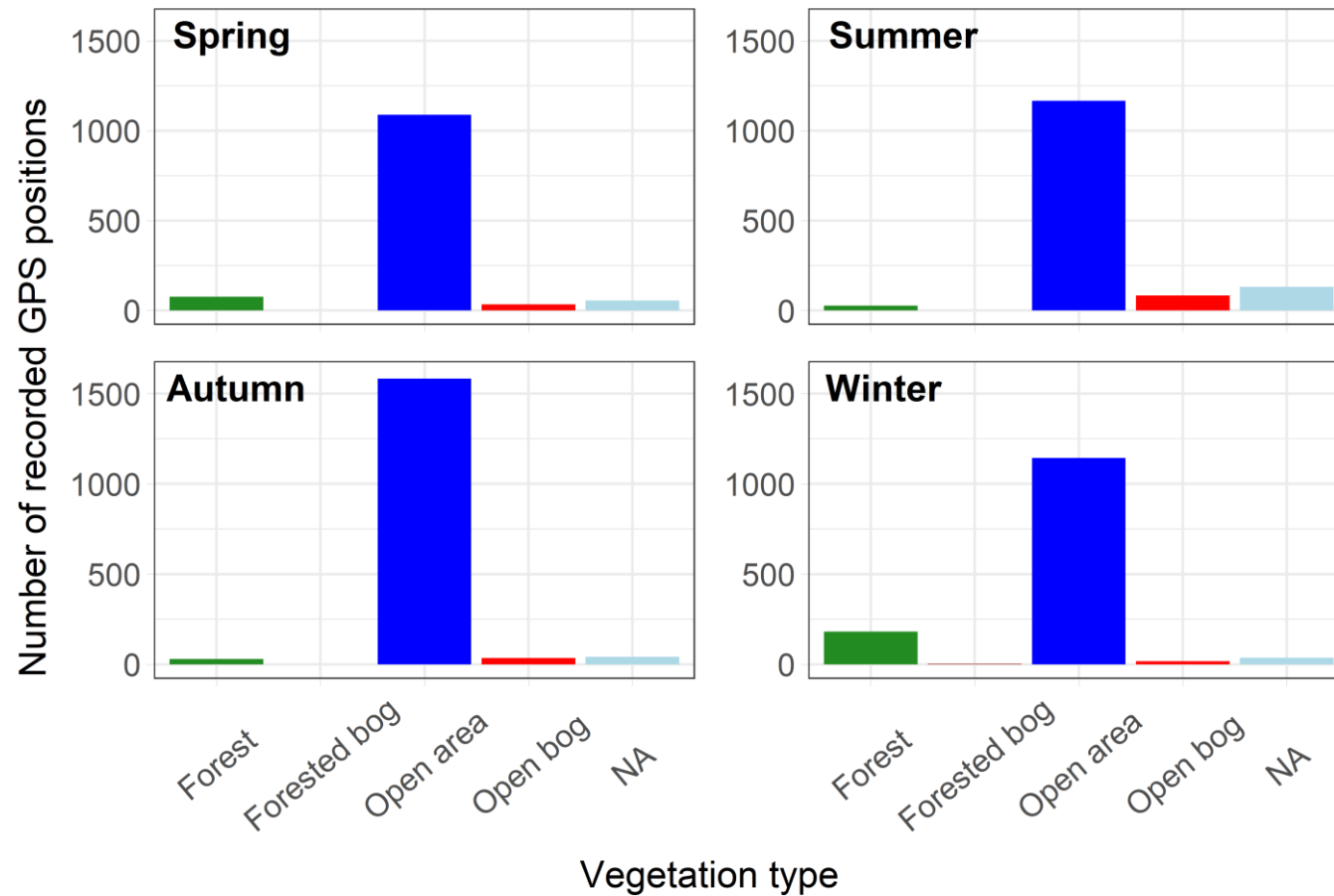


*Appendix 1 figure 1: Vegetation use of the northern reindeer group throughout 2019-2020. Spring = March - May, Summer = June - August, Autumn = September - November and Winter = December - February.*
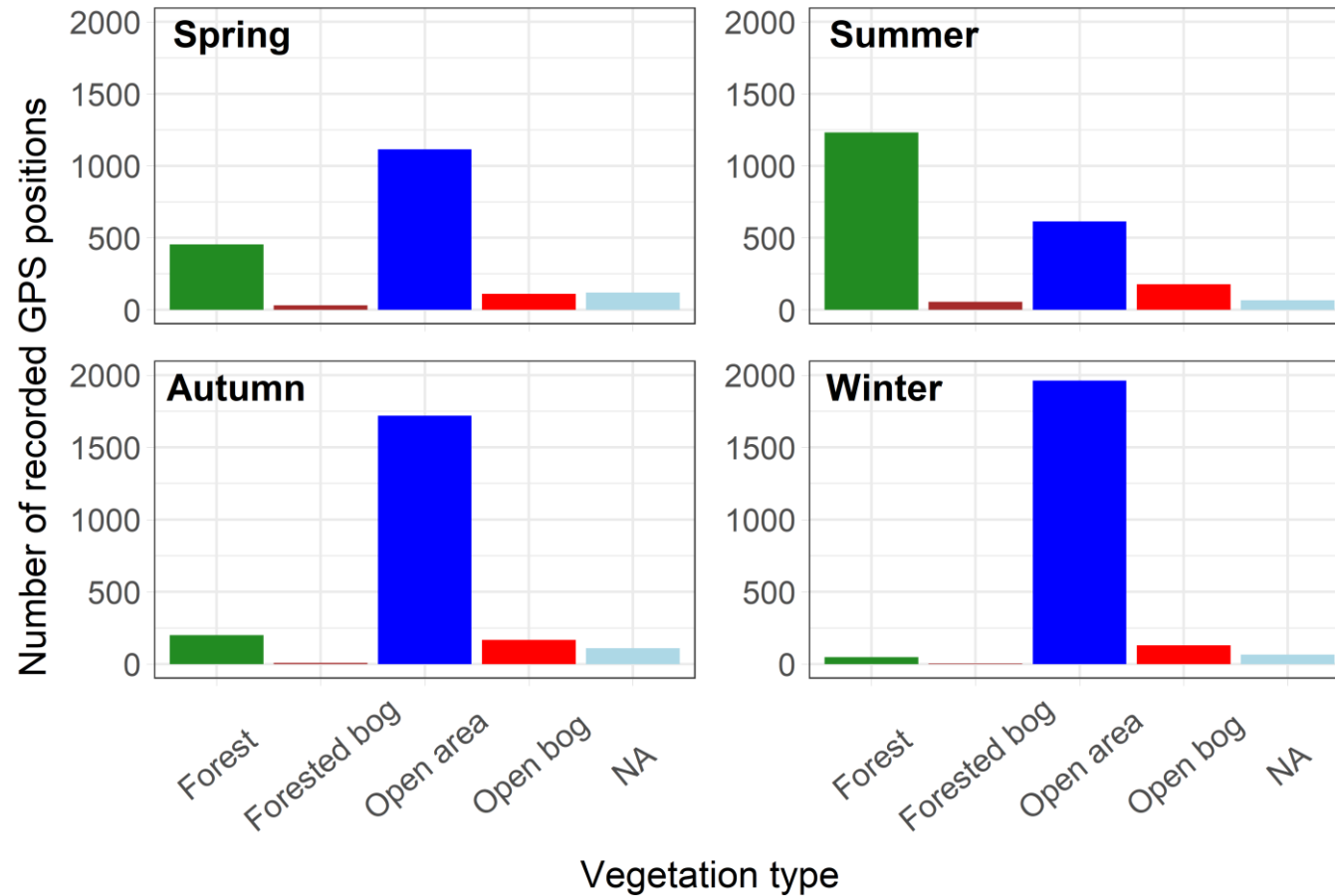
*Appendix 1 figure 2: Vegetation use of the southern reindeer group throughout 2019-2020. Spring = March - May, Summer = June - August, Autumn = September - November and Winter = December - February.*

# Appendix 2

*Appendix 2 table 1: Gastropod species abundance and composition in sampled 1x1m$^2$ plots in Rondane during weeks 35-37 in 2020.*

| | | | | Species | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Vegetation** | **Total plots** | **Plots with gastropods** | **Unidentified** | *Nesovitrea spp.* | *Punctum pygmaeum* | *Discus ruderatus* | *Cochlieopa lubrica* | *Arion subfuscus* | *Tandonia spp.* |
| Forest | 18 | 13 | 4 | 27 | 6 | 10 | 1 | 13 | 2 |
| Forested bog | 3 | 3 | 1 | 5 | 0 | 0 | 0 | 5 | 2 |
| Open area | 18 | 2 | 1 | 1 | 1 | 2 | 0 | 0 | 0 |
| Open bog | 10 | 2 | 7 | 0 | 7 | 0 | 6 | 2 | 0 |
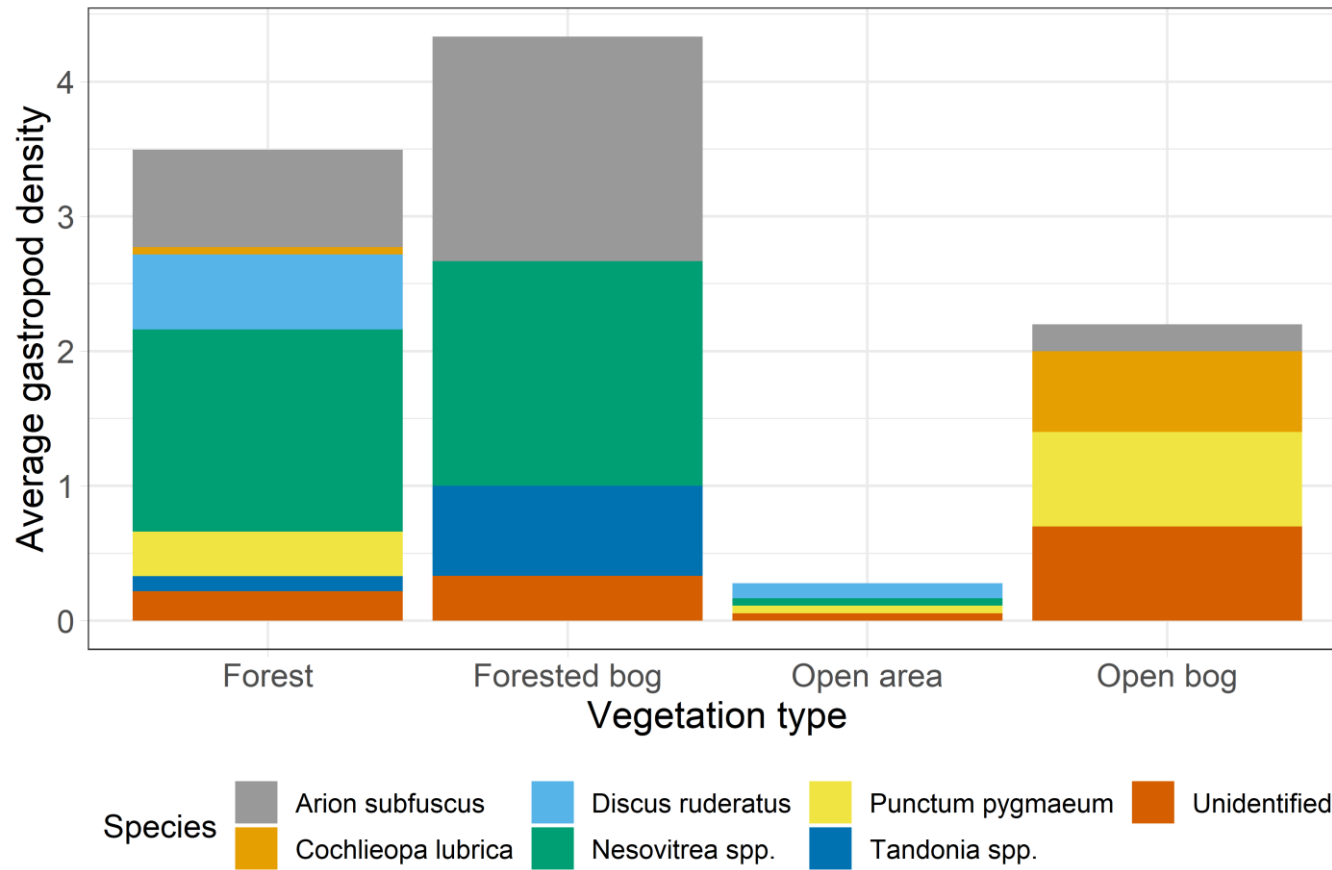
*Appendix 2 figure 1: Average gastropod density and species composition per plot (1x1m) for each sampled vegetation type in Rondane during weeks 35-37 in 2020.*