Coccidia in Svalbard rock ptarmigan as a health and population-regulating factor (RypeHOP)

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Figure 1. Svalbard rock ptarmigan in captivity. Photo: Renate Thorvaldsen

Summary

The Svalbard rock ptarmigan (*Lagopus muta hyperborea*) population has been monitored through spring territorial male counts since 2000 and autumn hunting statistics has been available since 1998. The ptarmigan is a low-density species and estimated spring densities of males indicate an increasing to stable population. The most important driver for this trend is increased winter temperature due to climate warming that may have improved winter survival.

However, the autumn hunting bag has been decreasing including a declining proportion of juveniles. Earlier studies identified changes in hunter effort as an explanation for reduced number of birds shot per year, but the cause of declining juvenile representation remained unknown.

As diseases and parasites can significantly influence wildlife population dynamics, this project investigated the role of parasites with particular focus on coccidia (*Eimeria* spp.) in ptarmigan health and assessed their potential as ecological indicators in a rapidly warming Arctic. The main objectives of this project were to evaluate if coccidia might contribute to reduced juvenile survival, which species of *Eimeria* might cause disease in ptarmigan and how rising Arctic temperatures might affect sporulation rates and infection risk.

Field faecal samples were collected from wild Svalbard rock ptarmigan in spring (April) and summer (June) 2024 and spring (April-May) 2025. In parallel, faeces from captive Svalbard rock ptarmigan at UiT The Arctic University of Tromsø (UiT) were analysed to track seasonal patterns and document clinical disease events. Faecal oocyst burdens (oocysts per gram, OPG) were quantified using a modified McMaster flotation method. Experimental work tested sporulation success and speed at different temperatures (5–40 °C) to model infection risk under warming conditions. Amplicon sequencing of the 18S rRNA gene was used to identify and compare *Eimeria* species across wild and captive hosts.

Key Findings

Coccidia are common in both wild and captive Svalbard rock ptarmigan although the prevalence was significantly higher in captive birds (89%) than in wild birds (58%) with egg flotation. Oocysts were detected in just three of the 12 summer samples from wild birds (prevalence 25 %). However, molecular results indicated the presence of *Eimeria* DNA in all summer samples and in all but one of the spring samples from wild birds. This would therefore indicate that the infection prevalence level was similar in the summer even though oocysts were not successfully visualized.

Wild birds reach infection levels known to cause disease in captivity. Several wild samples from key areas (Bjørndalen, De Geerdalen, Adventdalen, Revneset) exceeded 15,000 OPG, the threshold requiring medication to prevent clinical disease in captive birds. This supports the hypothesis that wild Svalbard rock ptarmigan may experience subclinical or clinical coccidiosis, potentially affecting juvenile survival.

Eimeria uekii was the dominant species and likely the main pathogen. Sequencing identified two species: *E. uekii* (dominant) and *E. rjupa* (to a lesser extent), with the occasional unresolved *Eimeria* sp. Outbreaks in captive birds were linked to *E. uekii*, indicating its pathogenic potential. Poultry vaccine strains were detected in a subset of captive chicks that had received the poultry vaccine prior to testing confirmed methodological sensitivity.

Sporulation success, based on the faecal samples from captive Svalbard rock ptarmigan, was strongly temperature dependent with optimal sporulation between 15–25 °C, with >90% of oocysts sporulating rapidly (within 1–3 days). However, sporulation occurred slowly but still successfully at 5 °C, indicating cold tolerance. There was also evidence of freeze tolerance, in the samples from wild Svalbard rock ptarmigan, with sporulation

occurring after short-term (1 week) but not long-term (9 months) freezing. Climate warming is hence likely to increase infection risk since rapid sporulation at ≥15 °C means that warmer summers and earlier springs could drive higher environmental parasite loads. This could disproportionately affect non-immune juveniles, potentially contributing to declining juvenile proportions in the hunting bag.

Findings

The findings demonstrate that:

- Wild Svalbard rock ptarmigan can carry *Eimeria* burdens high enough to cause disease.
- The predominant *Eimeria* species in the wild, *E. uekii*, is the same species implicated in clinical outbreaks in captivity.
- Temperature-sensitive sporulation suggests that Arctic warming may intensify parasite transmission dynamics.

Although the dataset remains limited, current evidence supports the possibility that coccidiosis may contribute to reduced juvenile survival. However, this data cannot yet quantify the extent to which coccidia contribute to long-term demographic trends.

Recommendations

- Establish long-term monitoring of coccidia prevalence, abundance, and species composition in wild Svalbard rock ptarmigan.
- Integrate coccidia metrics (abundance (OPG), prevalence, species identity) into existing ptarmigan monitoring programs as potential ecological indicators.
- Model climate-driven infection dynamics to predict future disease risk under continued Arctic warming.
- Conduct controlled pathogenicity trials to definitively determine species-specific virulence.

Conclusions

This study reveals that coccidia are widespread in Svalbard's rock ptarmigan and that environmental conditions under a warming Arctic climate may increasingly favour their transmission. While coccidia are unlikely to fully explain the declining juvenile proportion of the population, they represent a plausible contributing factor. Monitoring coccidian burden and species composition also offers a promising ecological indicator for assessing Svalbard's rock ptarmigan health and anticipating future population fluctuations.

Background

Annual population monitoring of the Svalbard rock ptarmigan (*Lagopus muta hyperborea*) has been conducted since 2000 by the Norwegian Polar Institute's monitoring programs, based on spring counts and hunting statistics since 1998 (COAT, 2024; Fuglei et al., 2023, 2020, 2019; MOSJ, 2023; Pedersen et al., 2012; Soininen et al., 2016). The Svalbard rock ptarmigan (Figure 2) is a low-density species that from 2000 varied between 1 to 3 males per km^2. From 2014 the spring densities of territorial males increased and now vary between 3-5 males per km^2 (Marolla et al., 2021) whilst annual hunting statistics (number of birds shot) and juvenile proportion (using age determination of wings) shows a negative trend (Fuglei et al., 2023, 2019). The most important driver of the recent ptarmigan population growth rate is increased winter temperature that lower metabolic costs, enabling increased winter survival (Marolla et al., 2021). This increased winter survival may compensate for the ongoing decline in the juvenile proportion (Fuglei et al., 2023). The decreased total numbers of birds hunted in autumn reflects changing hunter behaviour and not a declining population, based on socioecological hunter interviews (Fuglei et al., 2019; Soininen et al., 2016). The decreasing proportion of juveniles has been verified by comparing the wing hunting bag data to autumn counts of juveniles over a five year period (Fuglei et al., 2023, 2019). **The reason for the decline in the juvenile Svalbard rock ptarmigan proportion is still unknown** (Fuglei et al., 2023; Soininen et al., 2016).

Diseases and parasites can, together with many other factors like geographic contrasting food web contexts, influence population dynamics (Henden et al., 2017; Stenkewitz et al., 2016), however, no health assessments have, so far been carried out during the surveillance of the Svalbard rock ptarmigan. **Evaluating parasitic coccidia in wildlife populations can provide valuable insights into their health and their environments** (e.g. response to changes in climate and host population dynamics (Berto and Lopes, 2020; Stenkewitz et al., 2016; Henden et al. 2017). Coccidia are parasites that invade the intestinal tract, causing the disease coccidiosis. They can have a negative effect on host body condition, mortality and fecundity (Stenkewitz et al., 2016; Taylor et al., 2016) depending on the species of coccidia, infection site and infectious dose. Most animals develop a robust immunity to the parasite after exposure to infection, provided the infectious dose is low. However, with high infection pressure, or poor immunity, coccidiosis affect the growth and survival of young animals in particular (Stenkewitz et al., 2016; Taylor et al., 2016) and has previously been suggested to impact ptarmigan population dynamics (Brinkmann, 1927). Coccidia (*Eimeria* spp.) have been detected in rock ptarmigan in Japan, Iceland and on Svalbard (Matsubayashi et al., 2024, 2023; Nielsen et al., 2024; Stenkewitz et al., 2016) with seasonal variation in coccidia prevalence, abundance and species composition associated with population density effects (Stenkewitz et al., 2016). Two species of coccidia were recently documented in the Svalbard rock ptarmigan population; *Eimeria uekii* and *E. rjupa* (Matsubayashi et al., 2024; Nielsen et al., 2024).

The abundance of coccidia in faeces can be an indicator of disease risk as well as the level of oocyst contamination of the environment, from infected birds, and is thus a measure of infection risk for non-immune birds. Coccidia may function as ecological biomarkers for wild bird populations (Berto and Lopes, 2020). In Iceland, the density of rock ptarmigan was associated with the prevalence and aggregation of one coccidia species (*Eimeria muta*) with a 1.5-year time lag (Stenkewitz et al., 2016) with a negative association between *E. muta* prevalence and body condition as well as increased mortality and decrease fecundity in infected birds. If a similar lag is present in Svalbard rock ptarmigan, changing coccidia aggregation could potentially be used as an ecological indicator of population health and as an early warning system for population fluctuations. The mean peak abundance of coccidia in Icelandic ptarmigan (Stenkewitz et al., 2016) is similar to that seen during early clinical coccidiosis outbreaks in captive Svalbard rock ptarmigan (unpublished data, Norwegian Veterinary Institute, NVI). **Monitoring coccidia abundance can therefore indicate increased disease risk, especially for non-immune chicks and juveniles.**

Coccidiosis has been seen in captive rock ptarmigan populations in Norway (unpublished data, NVI) and Japan (Matsubayashi et al., 2024) with high morbidity and mortality. **Investigation of the coccidian species**

composition during clinical outbreaks in captive Svalbard rock ptarmigan should shed light on which of the species cause disease.

Coccidia eggs (oocysts) have to survive for a prolonged period in the environment after being shed in the faeces, before infecting a new host. During this free-living stage, the oocysts undergo further development, called sporulation. The time taken to sporulate depends on the *Eimeria* species and environmental factors like humidity and temperature (Graat et al., 1994; Waldenstedt et al., 2001). Different degrees of psychrotolerance have been found and shown in different species of cattle coccidia (*Eimeria* spp.) from the same region (Lassen and Seppä-Lassila, 2014). Thermal tolerance can also vary considerably between temperate and Arctic parasites of the same species (Davidson et al., 2008). However, little is known about the sporulation temperature and time combinations for coccidia of high-Arctic origin and whether a warmer climate would increase sporulation rate. Faster sporulation would lead to higher levels of infection in the environment. **Estimating sporulation times under different temperatures will provide insight to parasite infection dynamics under a changing climate.**

Objectives

The aims of this project were to investigate:

1. Whether coccidia could be causing reduced juvenile ptarmigan survival – as suggested by the decreasing proportion of juveniles in the autumn hunting bag.
2. If coccidia can be used as an ecological indicator for ptarmigan population health and as an early warning for population fluctuations.
3. Which species of rock ptarmigan coccidia cause disease.
4. How a warming climate might influence coccidia infection risk for non-immune juvenile birds.

Figure 2. Svalbard rock ptarmigan in captivity. Photo: Renate Thorvaldsen.

Materials and methods

Sampling and fieldwork

Captive Svalbard rock ptarmigan

UiT The Arctic University of Norway has Svalbard rock ptarmigan (originally collected from Svalbard, RiS ID 11781) in their research animal department (research facility approval number 089, Norwegian Food Safety Authority). The birds are housed either in pairs (male and female) or individually within 6 m² enclosures located in a roofed, open mesh-wire facility. Each enclosure is enriched with rocks, twigs, and sand baths, and the floors are covered with wood pellet bedding. Enclosures are cleaned at three-week intervals. The birds are provided *ad libitum* access to Lundi Grouse pellets (Lundi Farm, Germany). During the preparation for, and throughout, the breeding season, their diet is supplemented with small quantities of chicken pellets (Fryd Vekst/Fryd Kylling, Felleskjøpet), shell sand, and lingonberries (*Vaccinium vitis-idaea*). Additional enrichment is provided when available, including sorrel (*Rumex* spp.), willow (*Salix* spp.), and blueberry heather (*Vaccinium* spp.)

Captive ptarmigans (14 females and 7 males) were sampled and analysed, throughout summer 2024 to see if there were seasonal differences in parasite abundance, as well as to monitor for clinical disease outbreaks. In addition to this, four historical samples were also analysed: two pooled faecal samples from chicks when they arrived at the unit after being collected from Svalbard in August 2023 and again three weeks later.
Faecal samples were collected randomly during spring and summer to assess coccidia levels. Only freshly deposited, moist fecal pellets were collected to ensure sample quality. These were analysed for parasite egg and oocysts using the McMaster method described above, including the sieving steps for oocyst concentration. The concentrated oocysts were then stored frozen (-20 °C) until later molecular analysis.

For the sporulation trial, faecal samples were collected weekly from all the cages, early in the morning during routine checks to ensure freshness in August to September 2024. Samples were collected across all enclosures, with a minimum pooled sample size of 50 g per collection timepoint. These samples were placed in zip-locked bags that had had all the air squeezed out prior to sealing and then refrigerated until further analysis.

Wild Svalbard rock ptarmigan

Faecal samples were collected during three field sampling trips in Svalbard: Spring (April) and summer (June) 2024, and Spring (April-May) 2025, concurrent with other fieldwork carried out by the Norwegian Polar Institute.

Seventeen faecal samples were collected from the ground close to ptarmigan breeding territories in spring 2024, 12 in summer 2024 and a further 7 in spring 2025. The 2024 samples were frozen at -20 °C prior to analysis in January 2025, whilst the 2025 samples were stored chilled until analysis in May 2025. Six of the 2024 samples collected in the spring were relatively dry whilst the others contained more moisture. Most of the samples collected during the summer were dry and desiccated (N=10) whilst two were fresher and still had some moisture. The seven samples from spring 2025 were mostly dry but not completely desiccated.

Monitoring coccidia prevalence and abundance

Faecal samples were analysed using a modified McMaster egg flotation method based upon homogenizing 0.5-3 grams of faeces with 19-57 mL of tap water to ensure a detection sensitivity of approximately 20 oocysts per gram (OPG, Taylor et al. 2016). The homogenized faeces solution was then sieved (250 μm) and transferred to two conical falcon tubes up to the 10 mL mark. These were centrifuged at 1100 G for three minutes and the supernatant was discarded. A saturated sodium chloride glucose flotation fluid (specific weight 1.28) was added to the 10 mL mark in both tubes and vortexed to homogenised thoroughly. A 1 mL subsample was counted using

a Universal Whitlock chamber under 100 x magnification to estimate the number of oocysts present. Two chambers were counted (1 mL volume). The morphological appearance of the oocysts was noted, the total count transformed into oocysts per gram (OPG).

The OPG was calculated based on the following formula

$$OPG = oocyst\ count * (\frac{(Faeces\ weight\ +\ vol.\ water)}{(Faeces\ weight\ \cdot\ volume\ in\ chambers\ analysed)})$$

Both tubes were allowed to stand whilst the oocyst counting was carried out. Once the count was completed the top 2 mL of fluid from both tubes, regardless of the OPG, was placed in a sieve (nylon mesh size 21 μm) and rinsed with tap water before being flushed back into a 50 mL conical tube. Tap water was added to the 30 mL mark and the tube then centrifuged for 3 minutes at 1100 G. The supernatant was aspirated and the sediment carefully transferred to a cryotube (Thermo Scientific Nunc Cryotube) and frozen until later molecular analysis for species identification.

Sporulation trials

Captive Svalbard rock ptarmigan

The faecal samples from the captive Svalbard rock ptarmigan were kept refrigerated (4-8 °C) in zip-lock bags from collection until the start of the sporulation trial. The faeces in each bag was combined in one bag and the combined faecal matter was thoroughly mixed and homogenized by hand massaging. Approximately 10 grams were added to individual weighing boats (14×14×2.2 cm). A wet gauze with tap water, which was checked periodically to ensure it remained wet, and the labelled weighing boat containing the faeces were placed in an open plastic bag to allow aeration but prevent desiccation of the faecal material. The samples were incubated in incubators set to 5 °C, 10 °C, 15 °C, 20 °C, 25 °C, 30 °C, and 40 °C. At predetermined intervals (0, 1, 2, 3, 6, 10, 13 and 36 days), three 100–200 mg faecal samples were removed from each weighing boat and transferred into a 13 mL centrifuge tube. This was vortexed with 1 mL of flotation solution (Saturated NaCl solution (40% w/v, specific gravity 1.28) until fully dissolved. The tube was then filled to 10 mL with flotation solution and centrifuged at 387 rcf (1500 rpm) for three minutes. The top 1 mL of the supernatant was collected and loaded into two chambers of a Universal Whitlock McMaster counting slide. At least 100 oocysts were counted, after one minute to allow time for the oocysts to float, and the number of sporulated and unsporulated occysts were recorded. Unsporulated oocysts have a single undivided granulated mass as the dominant internal structure whilst in sporulated oocysts this mass has divided into four distinct structures (sporocysts) each containing two sporozoites. Three samples were analysed at each time point from each weighing boat to provide a technical triplicate for each temperature and time point combination.

The sporulation ratio for each sample was calculated using the formula:

$$Sporulation\ ratio\ (\%) = 100 * (\frac{No.\ sporulated\ oocysts}{Total\ no.\ oocysts\ observed})$$

The unsporulated versus sporulated ratio was plotted against temperature and logistic curves were fitted per temperature by pooling the technical replicates. The following parameters: onset time of sporulation (O_T), when sporulated oocysts were ≥5% of the total number; the time of 50% sporulation ($T_{50\%}$); and the maximum sporulation ($S_{MAX\%}$) were calculated for each temperature from the logistic fitted curve.

Wild Svalbard rock ptarmigan

The faecal samples from the wild Svalbard rock ptarmigan (spring and summer 2024) were kept frozen from time of sampling until the results of the sporulation trials from captive Svalbard rock ptarmigan samples had been finalized. This was done to select an optimal temperature combination for the subsequent sporulation trial for the wild Svalbard rock ptarmigan samples. The samples were thawed in January 2025 after a maximum of nine months of freezing. The faecal egg flotations were carried out using a modified McMaster method to estimate the individual oocyst burden, as described earlier. Only the spring 2024 samples were selected for the sporulation experiment. The faecal samples from summer 2024 were excluded from the sporulation experiment as they did not have high enough oocyst counts.

The remaining faeces from the spring 2024 samples were combined in one stomacher bag (total amount faeces 19.8 gram) and 5.04 mL of sterile distilled water added prior to homogenization for two minutes on the maximum setting in the stomacher (Seward Stomacher 80 BioMaster). The homogenized faeces was divided between three weighing ships (7 grams per ship) for incubation at 5 °C, 20 °C and 35 °C. The remaining 3.3 grams were placed in a fourth weighing ship and incubated at 20 °C. Each weighing ship was labelled with a unique identifier and placed in an open individual plastic bag containing wet gauze (7.5 x 7.5 cm gauze Mölnlycke Health Care). A subsample containing 100-200 mg of homogenized faeces was transferred to a labelled 15 mL falcon tube (VWR) at the following timepoints 0, 12 hours, 24 hours (day 1), 48 hours (day 2), day 3, day 6, day 7, day 9, day 10, day 13 and day 14. Samples were taken, where possible, in triplicate at each time point. One mL of flotation fluid was added to each falcon tube, vortexed to dissolve the faeces and additional flotation fluid added to the 10 mL mark. The flotation fluid was vortexed one more time and a 1 mL sample transferred to the Universal Whitlock chamber for evaluation and counting under 100 x magnification. A minimum of 100 oocysts were assessed, or up to a minimum of four counting chambers per sub-sample, whichever was reached first. Once a sample contained fewer than 50 oocysts in four counting chambers, or there was no more faeces, the sample was excluded from further investigation.

The faecal samples from the seven wild Svalbard rock ptarmigan faecal samples collected in spring 2025 were individually analysed for OPG prior to being combined in a stomacher bag and homogenized in the stomacher for two rounds of two minutes on the highest setting, without the addition of sterile water (Figure 3). The samples were then divided equally between four weighing ships, and each weighing ship was individually vacuum packed. Two of the vacuum packs were labelled 1a and 1b, whilst the other two were labelled 2a and 2b. The two vacuum packs labelled 1 were placed in the fridge (5 °C) and the ones labelled 2 were placed in the freezer (-20 °C) for 7 days prior to carrying out the sporulation trials.
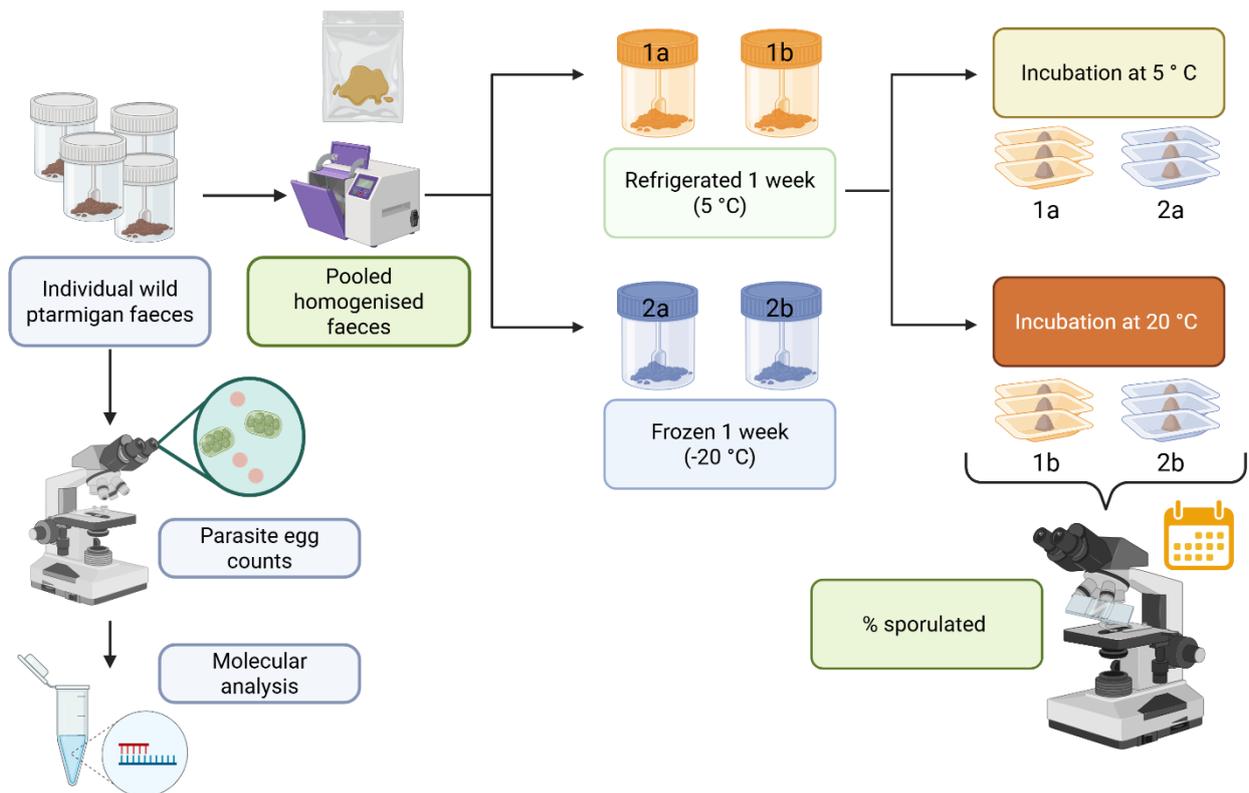
Figure 3. Sample analysis and study design for the sporulation experiment with pooled wild Svalbard rock ptarmigan faeces from spring 2025 as well as the individual egg counts using McMaster analysis for wild Svalbard rock ptarmigan samples from 2024 and 2025. Figure made in BioRender.

After 7 days the vacuum sealed samples were removed from storage and opened. A wet gauze compress was placed underneath the weighing ship and the bags then placed in either the fridge 5 °C (labelled a) or into the incubator (labelled b) at 20 °C. Three sub-samples from each weighing ship (100-200mg) were transferred to three individual labelled 15mL falcon tubes at each time point and these were placed in a rack, with the lid on, in the fridge until flotation could be carried out. One tube at a time was removed from the fridge and 1 mL saturated sodium chloride solution with glucose (specific weight 1.28) was added to the tube, vortexed until the faeces had dissolved and then the tube topped up to the 10 mL mark before transferring the homogenized fluid to a Universal Whitlock chamber for counting. The first 100 oocysts or 4-8 chambers were counted depending on which came first. The counting was stopped after 8 chambers even if the total number of oocysts was still less than one hundred. The number of sporulated and unsporulated oocysts were counted separately. If there was any doubt regarding sporulation, the oocyst was interpreted as not sporulated.

Coccidia species identification

DNA extraction

DNA was extracted from wild Svalbard rock ptarmigan samples collected in the summer (2024, n=12), samples collected in the spring (2024 and 2025, n=17) and samples from captive Svalbard rock ptarmigans (2024, n=16). The samples contained oocysts that were floated and purified from faeces using an in-house protocol at NVI developed for the TEiCON project (FFL/JA 310711). The QIAamp PowerFecal Pro DNA Kit (Qiagen, Hilden, Germany) was used following the manufacturer's recommendations with an automated protocol on the Qiacube connect robot (Qiagen, Hilden, Germany). The samples were centrifuged at 15000 x g for two minutes before the supernatant was removed. The oocysts were resuspended in 800 µl CD1 buffer from the kit and the samples

were transferred to PowerBead Pro tubes (Qiagen, Hilden, Germany). In addition, a negative control (empty tube) and a positive control (100 µl Paracox-5, MSD Animal Health) were included. The samples were homogenized using FastPrep-24™ 5G (MP Biomedicals, Irvine CA, USA) in 3 rounds x 60 sec at 5 m/s with 5 min rest between rounds. The samples were centrifuged at 15000 x g for 1 min and DNA from 500 µl of the supernatant was extracted according to the PowerFecal DNA pro IRT protocol on the Qiacube and eluted in 50 µl. The concentration of the eluted DNA was measured by the Qubit BR assay (Thermo Fisher Scientific, Waltham, MA, USA) following the manufacturer's recommendations. The eluted DNA was stored at -20 °C.

18S rRNA amplicon sequencing

To investigate which *Eimeria* species were present in the samples, the 18S rRNA gene was amplified by PCR and sequenced according to recommendations from Illumina. The amplicon PCR was performed including the forward primer 5'-CGCGCAAATTACCCAATGAA-3 and the reverse primer 5'-ATGCCCCCAACTGTCCCTAT-3' (Hinsu et al., 2018). The forward and reverse primers were tailed with partial Illumina adapters: ACACTCTTTCCCTACACGACGCTCTTCCGATCT and GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT, respectively. Spacers were inserted between the gene specific sequence and the adapter (de Muinck et al., 2017). The primers with six different spacers were mixed prior to PCR. The PCR was performed with the KAPA HiFi PCR Kit (Roche, Basel, Switzerland) on a Thermal Cycler (Thermo Fisher Scientific, Waltham, MA, USA) with the following cycling conditions: initial denaturation at 95 °C for 3 min, 34 cycles of 98 °C for 20 s, 65 °C for 30 s, 72 °C for 30 s, and one cycle of 72 °C for 10 min. Ten samples from the captive Svalbard rock ptarmigans was amplified with annealing temperature for 60 °C instead of 65 °C. The PCR products were purified with AMPure XP beads (Beckman Coulter Life Sciences, Brea, CA, USA) before the indexing PCR. The same PCR kit was used with the following PCR program; initial denaturation at 95 °C before 8 cycles of 98 °C for 30 s, 55 °C for 30 s and 72 °C for 30 s followed by one cycle of 72 °C for 5 min. The PCR products were again purified with AMPure XP beads, quantified with a D1000 TapeStation kit on a 4200 TapeStation System (Agilent, Santa Clara, CA, USA) and pooled in equimolar concentrations into one library for the ten samples from captive Svalbard rock ptarmigans and one library for the other samples. The library pools were sequenced on separate V3 flowcells with 2 x 300 bp chemistry on the Illumina MiSeq.

Bioinformatics analysis

The raw sequencing reads (the individual DNA sequence fragments generated by a sequencing run) were received demultiplexed and the sequences were bioinformatically analyzed by the Nextflow Ampliseq pipeline version 2.14.0 (Straub et al., 2020). The pipeline includes primer removal, quality check, trimming, error correction, merging, chimera removal and construction of the ASV (Amplicon Sequence Variant) table. In addition, clustering to Operational taxonomic units (OTUs) was included at 99% similarity. Taxonomy was added down to species level to each OTU using the PR2 - Protist Reference Ribosomal Database - Version 5.0.0 for Eukaryotes (Guillou et al., 2013). OTUs assigned to *Eimeria* without complete taxonomic species assignment were further classified using NCBI blast (Altschul et al., 1990).

Statistical analysis

JMP statistical software (version 19.0.4 JMP Statistical Discovery LLC) was used to analyse the *Eimeria* abundance data and sporulation trial results (sporulation percentage). The abundance data (OPG) between the two population (captive and wild), geographic origin and month of sampling was compared using contingency analysis and non-parametric analysis (Kruskal-Wallis tests) given the overdispersion of the data. The sporulation data was analysed using contingency analysis comparing storage and incubation temperatures with percentage sporulated at different time points and non-linear logistic regression curves were constructed to investigate onset to sporulation (time until ≥5% first sporulation (O_T), time to 50% sporulation (T_{50}) and maximum sporulation (S_{max}, asymptote of logistic regression curves). The inverse prediction comparison function was then

used to estimate the time to 5 % (O$_T$) and 50 % (T$_{50\%}$) sporulation. A significance level of p<0.05 was selected for all statistical analyses.
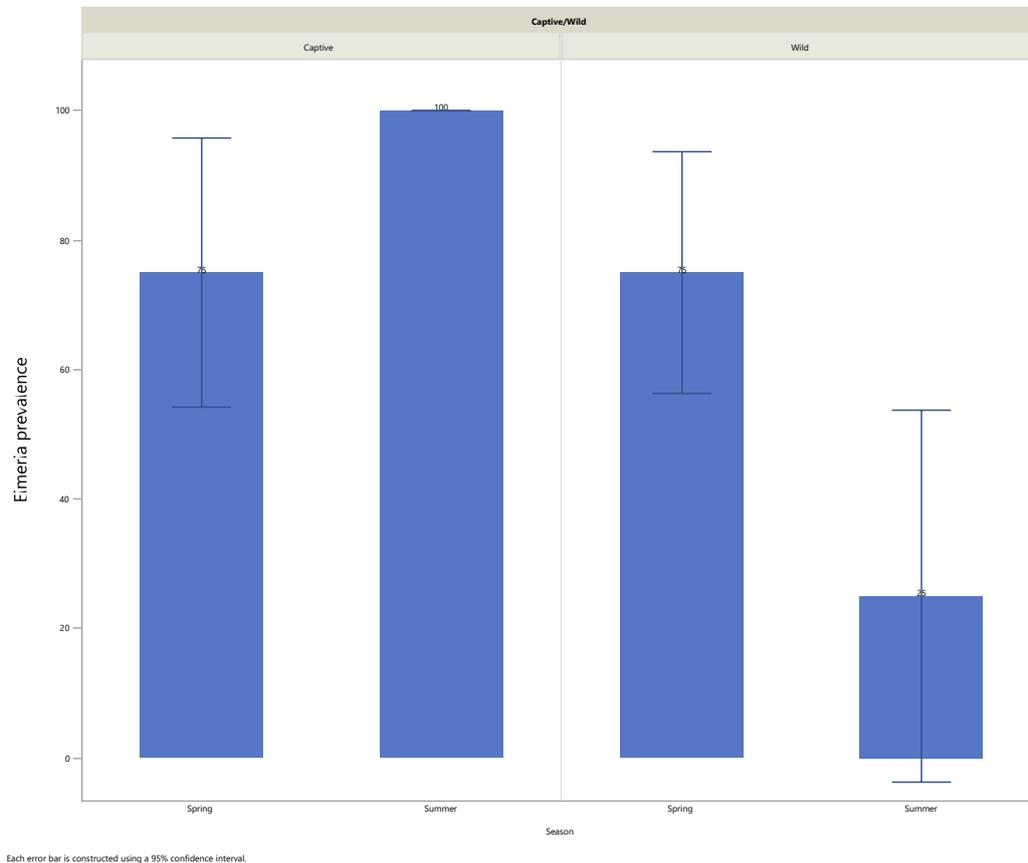
Results

Eimeria spp. prevalence

Figure 4. The mean seasonal prevalence (%) of *Eimeria* spp. oocysts in captive (n=47) and wild (n=36) Svalbard rock ptarmigan faeces sampled in 2023 (n=4 captive), 2024 (n=47 captive, n=29 wild) and 2025 (n=7 wild) with the error bar showing the 95 % confidence interval for the mean prevalence.

A total of 83 faecal samples were analysed during the study from wild (N=36) and captive (N=47) Svalbard rock ptarmigans spanning from April to September in 2023, 2024 and 2025. Wild rock ptarmigans had significantly lower mean *Eimeria* oocyst prevalence (58% [42-73 95 % CI]) than captive (89% [77-95 95% CI]) rock ptarmigans (p<0.01; Table 1; Figure 4). No other parasite eggs were detected. Only three of the samples from the wild Svalbard rock ptarmigans that were sampled in the summer had detectable oocysts on faecal egg flotation (N=12), whilst 75% of both the wild (18/24) and captive (15/20) ptarmigans sampled in the spring (in April and May) had *Eimeria* oocysts in their faeces (Figure 5).
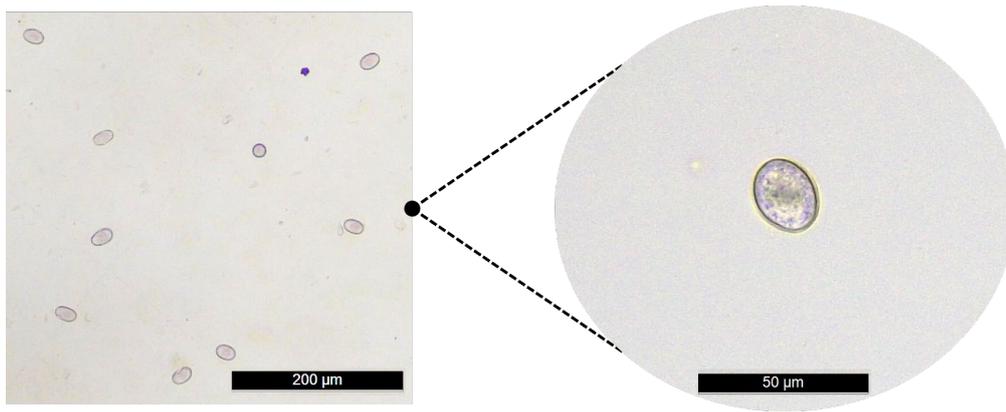
Figure 5 Unsporulated *Eimeria* oocysts from Svalbard rock ptarmigan seen during McMaster analysis. Oocysts with both oval and round morphology can be seen in the flotation fluid.

There was a significant increase in *Eimeria* prevalence in the captive Svalbard rock ptarmigan population (p<0.01) from April (50%; N=10) to the subsequent months (100%, N=37)). There was no significant difference in prevalence between the different wild Svalbard rock ptarmigan sampling areas (p=0.12) although birds sampled from Adventdalen had the highest *Eimeria* infection prevalence followed by those from Bjørndalen (Figure 6) if the single positives from De Geerdalen, Grønnsteinfjellet, Isdammen and Sassendalen are not taken into consideration given their limited sample size. Likewise for the areas with no *Eimeria* detected – here too only single samples were obtained.
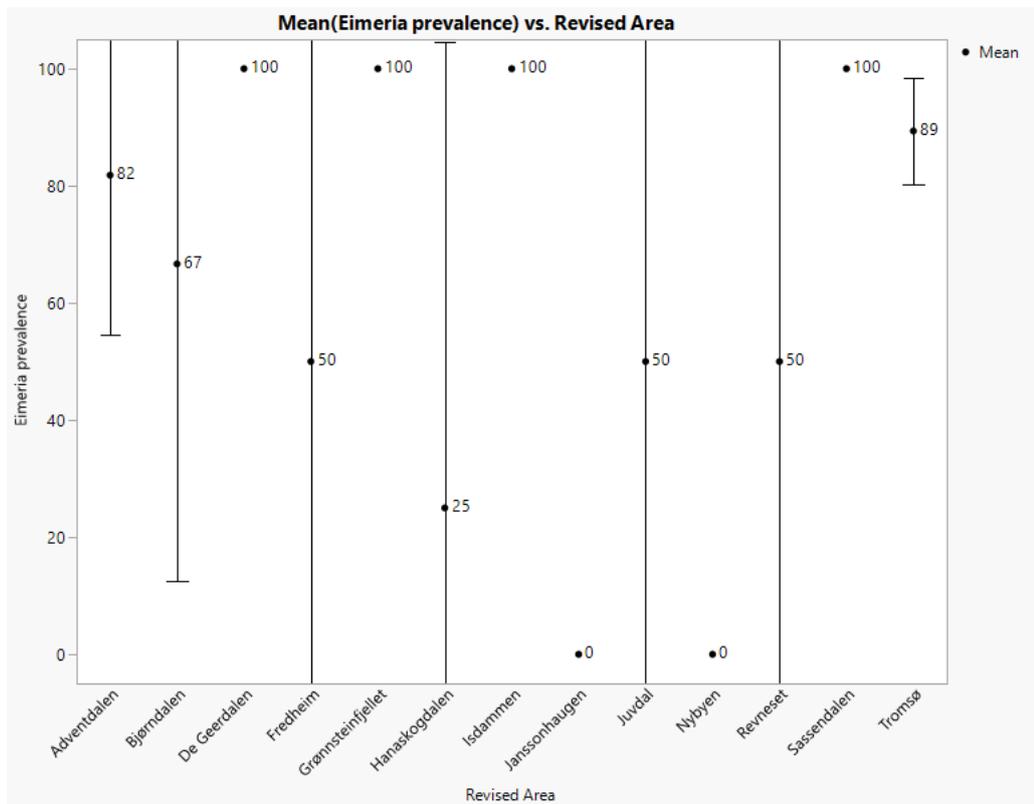

Figure 6. Mean *Eimeria* oocyst prevalence (%) in wild Svalbard rock ptarmigan faeces (n=34) at different sampling localities on Svalbard (Adventdalen n=11; Bjørndalen n=6; Hanaskogen n=5; Fredheim, Juvdal og Revneset n=2; De Geerdalen, Grønnsteinfjellet, Isdammen, Janssonhaugen, Nybyen, Sassendalen n=1) and in captive Svalbard rock ptarmigan faeces in Tromsø (n=47). At some locations only single individuals were sampled and so the 95 % confidence interval is not shown. The locality was not recorded for two of the wild samples so this data is not shown.

Eimeria spp. abundance

The abundance data (OPG) from both populations was left-skewed with a high number of negative samples or samples with very low coccidia abundance levels (Table 1, Figure 7). There were no significant differences in mean *Eimeria* spp. OPG between the two populations (p=0.79), but the median OPG was significantly lower in the wild population compared to the captive (p<0.05).

Table 1. The results from wild and captive Svalbard ptarmigan faecal oocyst counts using a modified McMaster flotation method including the 95% confidence interval (CI) for the prevalence and mean abundance (oocysts per gram, OPG).

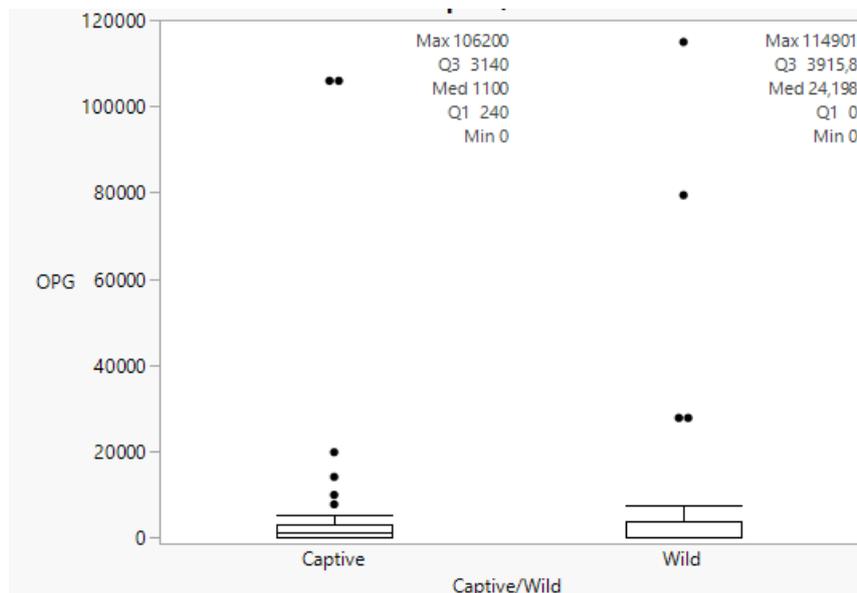| Ptarmigan population	N	*Eimeria* spp. prevalence (%) [95% CI]	Mean *Eimeria* spp. OPG [95 % CI]	Median OPG	Range
Wild	36	58.3 [42.2-72.9]	8049.8 [184.1 - 15 915.4]	24.2	0 - 114 901
Captive	47	89.4 [77.4-95.4]	6728.7 [427.3 - 13 030.2]	1100	0 - 106 200

Figure 7. Box whisker plot of the abundance of *Eimeria* spp. oocysts in Svalbard rock ptarmigan faeces in the captive (n=47) and wild (n=36) birds sampled in 2024 and 2025 showing the interquartile range, mean, median and outliers.

Although captive Svalbard rock ptarmigan showed a slight trend of increasing *Eimeria* abundance (OPG) from April to September the significance level was not reached with monthly (p=0.47) comparisons nor with seasonal comparison (p=0.17; spring (April-May); summer (June-September), Figure 8). Ten cages a month were sampled in the captive population, however different cages could be sampled each month. Some were only sampled once whilst others were sampled monthly, making further comparison of the trends challenging. Cage 16, in which a coccidiosis disease outbreak occurred, had higher OPGs than the other cages although the significance level was not reached given the small sample sizes for each cage (p=0.14). The birds in this cage were given Dozuril 50mg/ml, 2 ml/L in drinking water for 7 days. Similar analysis of the wild Svalbard rock ptarmigan comparing samples collected in the spring (N= 24, April and May) and summer (N=12, August) found no significant difference in abundance between these two periods (p=0.14).
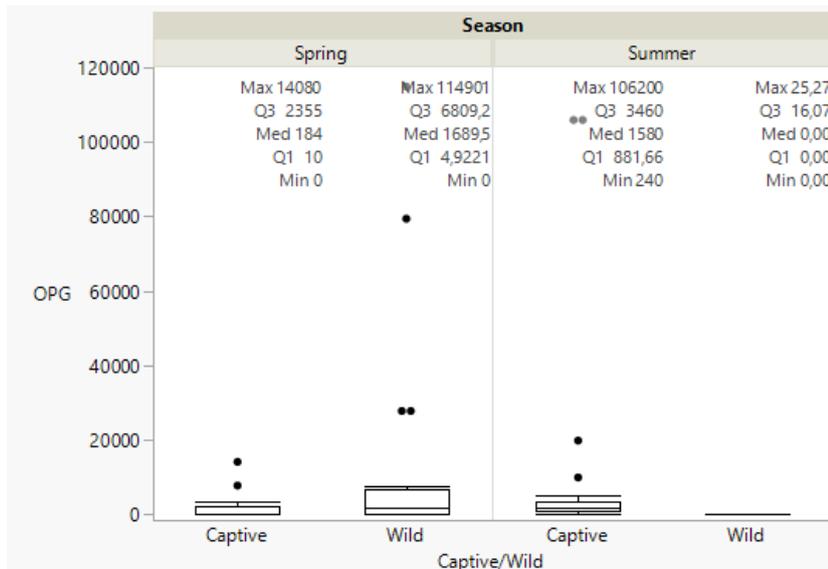
Figure 8. Box whisker plot showing the interquartile range, mean and outliers of Eimeria spp. oocyst abundance (oocysts per gram: OPG) in Svalbard rock ptarmigan faeces sampled in spring (April-May) and summer (June-September) in wild (spring n=24, summer n= 12) and captive (spring n=20, summer n=27) bird populations

Geographic differences in abundance were also seen in the wild Svalbard ptarmigan samples (p<0.01; Figure 9). However, small sample sizes (1-1 samples per location), especially the single outlier in De Geerdalen, had a disproportionate effect on the analyses. The single sample from De Geerdalen had a significantly higher OPG than all other areas (p<0.01). No other significant differences in OPG were seen by geographic origin.
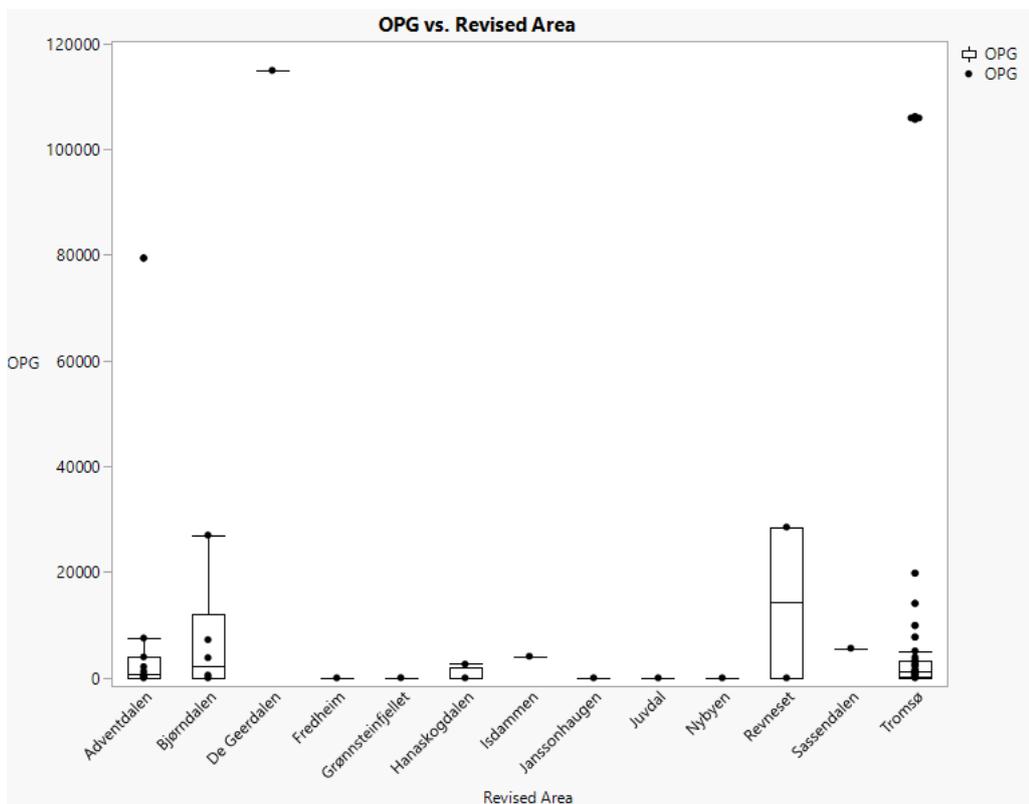

Figure 9. Box whisker plot showing the interquartile range, mean and outliers of *Eimeria* spp. abundance (oocysts per gram, OPG) by sampling locality of wild (Adventdalen n=11; Bjørndalen n=6; Hanaskogen n=5; Fredheim, Juvdal og Revneset n=2; De Geerdalen, Grønnsteinfjellet, Isdammen, Janssonhaugen, Nybyen, Sassendalen n=1) and captive Svalbard ptarmigan (Tromsø n=47).

Sporulation trials

Captive Svalbard rock ptarmigan

This experiment examined the sporulation dynamics of coccidia oocysts under temperatures ranging from 5 °C to 40 °C, with time-points extending up to 36 days. Logistic curve fitting was applied to describe sporulation kinetics at each temperature (Table 2; Figure 10). Logistic fits succeeded for all temperatures but 40 °C. The sporulation onset time (O_T) at low temperatures (5-10 °C), was delayed typically appearing between 10–13 days. At intermediate temperatures (15–25 °C), sporulation began much earlier, between 1–3 days, indicating the area of optimal temperature for metabolic activity. At 30 °C, O_T occurred rapidly (<2 days), but higher temperatures (≥30–40 °C) inhibited sporulation almost entirely. $T_{50\%}$ generally decreases with increasing temperature (faster sporulation at higher temperatures), but $T_{50\%}$ and temperature were not corelated linearly which indicates that the effect of temperature on sporulation is not straight forward dose dependent. Maximum sporulation percentages $S_{MAX\%}$ were mostly high, approaching 90–100% between 15 °C and 30 °C, dropped notably below 10 °C and was 0 % at 40 °C.

Table 2. Summary table from the rock ptarmigan *Eimeria* sporulation experiment that investigated time to onset of sporulation (O_T) calculated as the number of days until sporulation was >5%; the time until 50% sporulation ($T_{50\%}$ (Days)) and the maximum percentage of sporulation ($S_{MAX\%}$) at each temperature tested (T(°C)). The O_T and the $T_{50\%}$ for the 40 °C temperature are marked with X as no oocysts sporulated at any time point at this temperature.

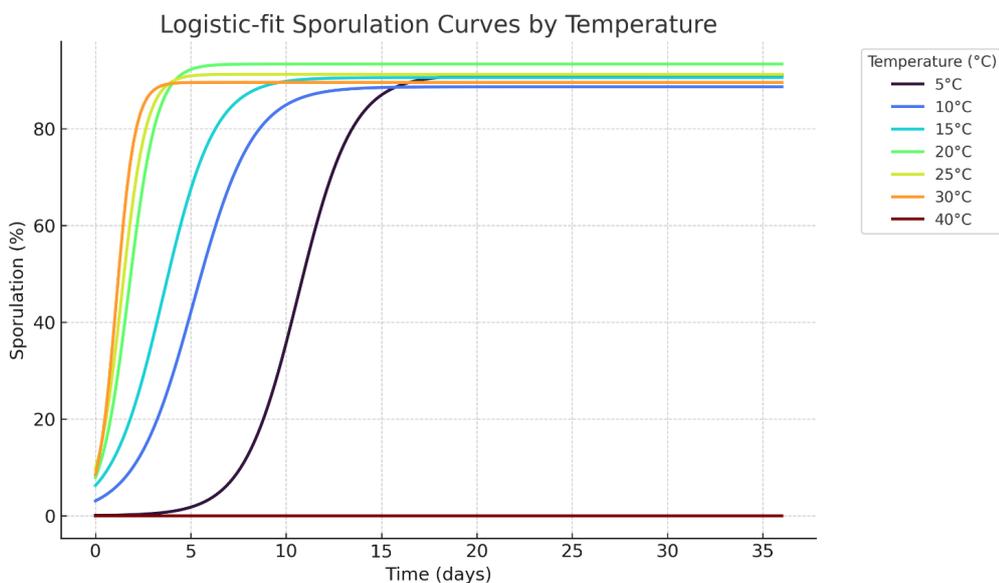| T(°C)	O_T (Days)	$T_{50\%}$ (Days)	$S_{MAX\%}$
5	6.5	10.9	91.2
10	0.8	5.6	88,7
15	0.3	3.8	90,6
20	0.1	1.8	93,4
25	<0.1	1.5	91,3
30	<0.1	1.21	89,6
40	X	X	0

Figure 10. Logistic-fit curves plotting the progression of *Eimeria* sporulation over time in relation to the incubating temperature for oocysts obtained from captive Svalbard rock ptarmigan.

Wild Svalbard rock ptarmigan

None of the samples that had been stored frozen for up to 9 months, from April 2024 to January 2025 prior to entering the sporulation experiment, sporulated during the two-week trial at any of the three incubation temperatures (5, 20 and 35 °C).

The samples collected in spring 2025 successfully sporulated, to varying degrees, after being stored frozen or chilled (1 week) and subsequently incubated at either 5 or 20 °C for up to two weeks. The *Eimeria* oocysts sporulated rapidly in the 20 °C incubator regardless of prior storage regime (Table 3; Figure 11). There was no significant difference in sporulation percentage between the two storage groups (frozen and fridge) when incubated at 20 °C (p=0.61). There was a significant difference in sporulation percentage, however, between the two incubation temperatures with samples incubated in the fridge having significantly lower sporulation percentages than those incubated at 20 °C (p<0.001). Sporulation still occurred however, albeit more slowly, in the samples incubated at fridge temperatures. There was also a significant difference in sporulation percentage between the frozen and chilled storage groups at fridge incubation temperatures (p<0.01).

Sample storage	N	Incubation temperature	Mean sporulation % [95 % CI]	Median sporulation %	O_T (days)	$T_{50\%}$ (days)	Range sporulation %	$S_{max\%}$
Fridge (5 °C)	20	Fridge (5 °C)	17.6 [7.8-27.4]	8.0	0.3	12.7	0-65.4	90.3
Freezer (-20 °C)	21		4.7 [2.6-6.7]	4.0	3.6	NA	0-18.2	7.7
Fridge (5 °C)	21	Incubator (20 °C)	60.0 [47.6-72.3]	66.2	0.5	0.95	0-91.1	72.3
Freezer (-20 °C)	21		55.9 [44.4-67.4]	62.5	0.5	0.85	0-84.8	66.2

Table 3. The mean, median and maximum ($S_{max\%}$) sporulation percentage (%) for the entire experimental period, onset time (O_T) until 5 % sporulated and time to 50% ($T_{50\%}$) sporulation in days, of *Eimeria* collected from wild rock ptarmigan on Svalbard during the 14 day sporulation trial after two different storage and incubation temperatures combinations. N = total number of subsamples analysed during the trial.
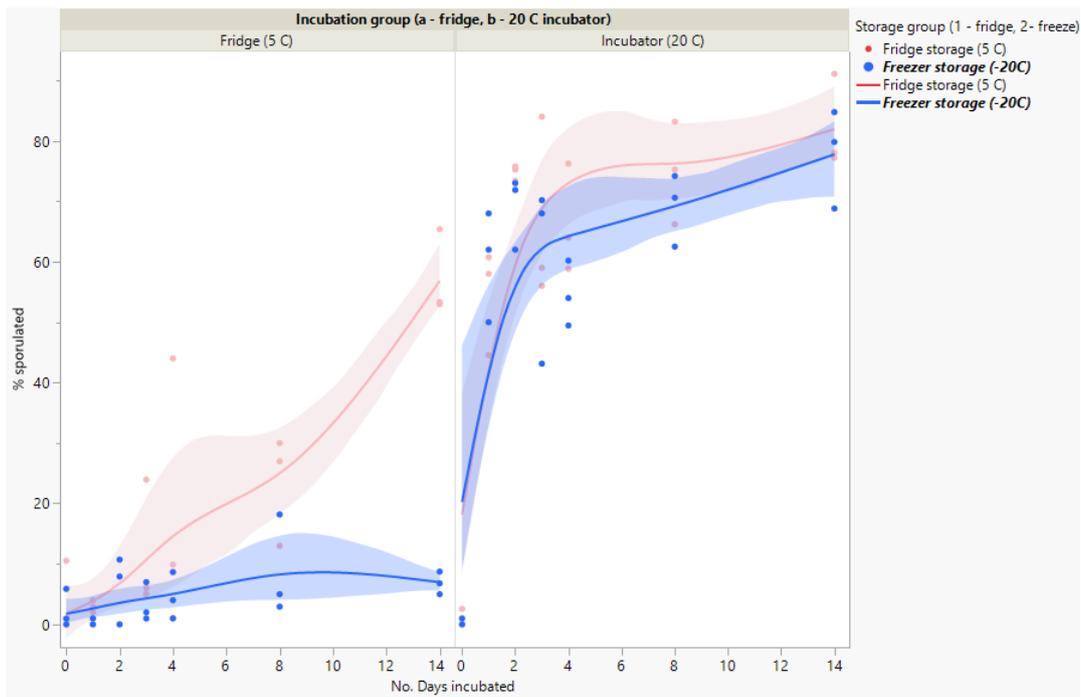
Figure 11. The percentage of sporulated Eimeria oocysts from homogenised wild Svalbard rock ptarmigan faeces, in each sample group analysed in triplicate on day 1-5, 8 and 14 post-incubation. Samples were either stored in the fridge or freezer for 1 week prior to incubation 5 °C (in the fridge) or at 20 °C (in an incubator). The mean sporulation percent for each time point shown by the solid blue (for those stored in freezer) and red (for those stored in fridge) prior to incubation as well as the 95% confidence interval for this line shown by the shading.

Coccidia species identification

The samples that were sequenced for species identification included 12 samples collected from wild Svalbard rock ptarmigans in the summer, 17 samples collected from wild Svalbard rock ptarmigans in the spring and 16 samples from captive Svalbard rock ptarmigans.

There was a difference between the seasons with OPG numbers ranging from 0-25 in the summer and 0-114901 in the spring, with a corresponding higher number of reads in the spring. The OPG numbers from the captive Svalbard rock ptarmigans ranged from 0-106200. The results are presented in Table 4. There were differences in the number of reads between the captive and wild population despite having similar mean OPG levels in the spring samples from the wild population and the captive population. However, there were slight methodological differences in the DNA sequencing, as the samples were sequenced in two separate runs with different numbers of samples per flow cell, which can account for this.

Table 4. Number of *Eimeria* reads and oocysts per gram (OPG) faeces in the sequenced samples from captive Svalbard rock ptarmigans and wild Svalbard rock ptarmigans from summer and spring 2024. *Only OPG numbers for four samples. N = number of samples. Reads = the number of individual DNA sequence fragments generated by a sequencing run. IQR = Interquartile Range, which is a measure of statistical dispersion.

Sampling season	Population	N	Mean reads [min-max] Median *IQR*	Mean OPG [min-max] Median *IQR*
Summer	Wild	12	11547 [336-38866] **5158** *16750*	6 [0-25] **0** *5.25*
Spring	Wild	17	36074 [673-86404] **36761** *36011*	14434 [0-114901] **513** *7213*
Summer	Captive	6*	62131 [27224-141859] **47204** *37619*	34645 [2680-106200]* **14850** *33305*
Spring	Captive	10	306224 [59-997345] **205946** *487470*	66 [0-360] **20** *62*

From the first sequencing run, after removing OTUs representing the positive control and species not assigned to *Eimeria*, the top 10 most abundant OTUs accounting for 96% of the total reads were further investigated by NCBI blast (Altschul et al., 1990). The results showed that five OTUs, including the two most abundant OTUs, could be assigned to *E. uekii.* One OTU, which was the third most abundant, was assigned to *E. rjupa* (Matsubayashi et al., 2024; Nielsen et al., 2024)*. The other OTUs could not be clearly assigned to a single *Eimeria* species and were therefore assigned as *Eimeria sp*. The results are presented in Table 5. Figure 12 shows the prevalence (% of total number of reads within samples) of the top 10 OTUs assigned to either *E. uekii, E. rjupa* or *Eimeria sp.* in the samples from the wild Svalbard rock ptarmigans from the summer (A) and the spring (B).

Table 5. BLAST hits for representative sequences from the top 10 OTUs from the first sequencing run assigned to *Eimeria* from both wild and captive Svalbard rock ptarmigan faecal samples. The table includes OTU ID, number of reads per OTU, accession numbers of matched reference sequences, species, percentage identity, e-values, and other relevant alignment metrics.

| OTU_ID	Reads	% of total reads	Accession nr	Species	% identity	E-value	Bit score
OTU 1	503270	44.8	LC380052	*Eimeria uekii*	99.8	0	763
OTU 2	380756	33.9	LC380046	*Eimeria uekii*	100	0	769
OTU 3	125008	11.1	LC756964	*Eimeria rjupa*	100	0	721
OTU 4	31327	2.8	NA	*Eimeria* sp.	NA	NA	NA
OTU 5	12839	1.1	NA	*Eimeria* sp.	NA	NA	NA
OTU 6	9484	0.8	LC380052	*Eimeria uekii*	97.8	0	719
OTU 7	8340	0.7	LC380052	*Eimeria uekii*	97.8	0	719
OTU 8	6578	0.6	LC380052	*Eimeria uekii*	97.1	0	702
OTU 9	1537	0.1	NA	*Eimeria* sp.	NA	NA	NA
OTU 10	701	0.1	NA	*Eimeria* sp.	NA	NA	NA
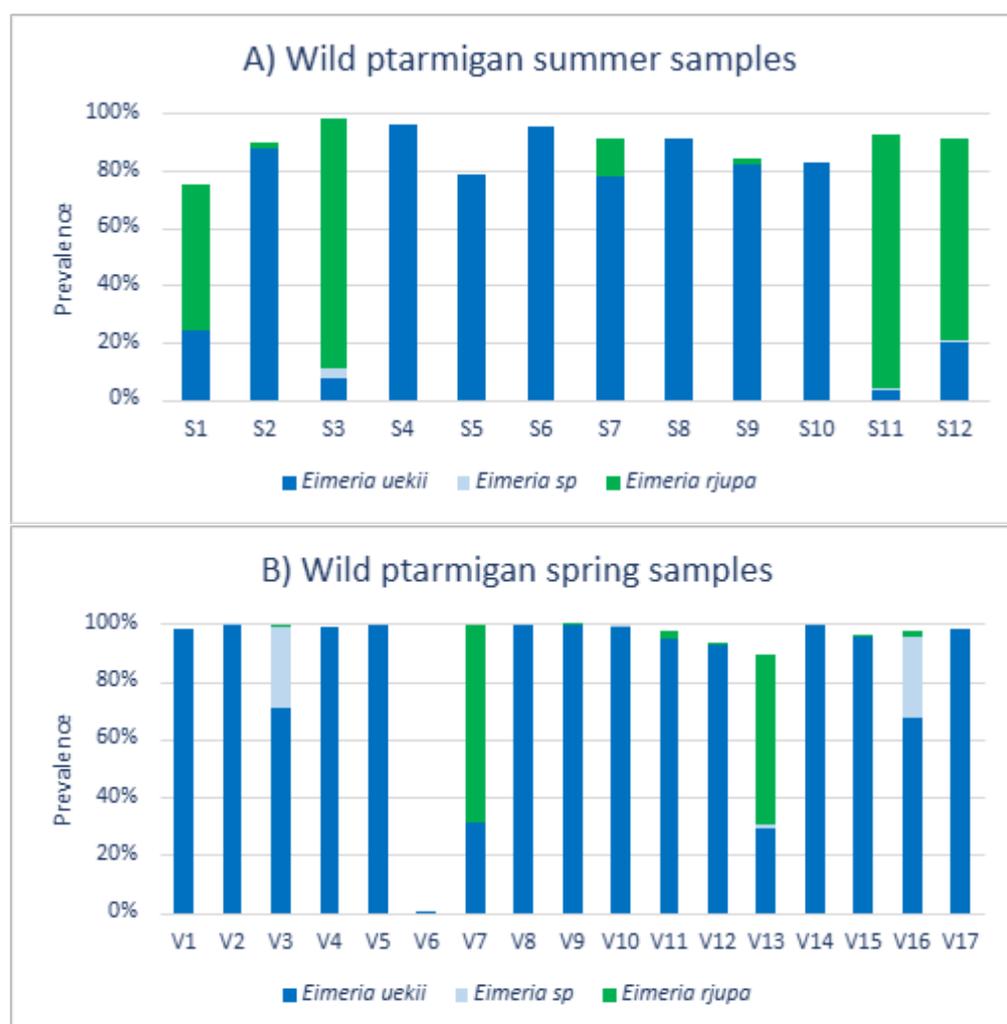
Figure 12. The prevalence (% of total number of reads within samples) of the top 10 OTUs assigned to either *Eimeria uekii, Eimeria rjupa* or *Eimeria sp.* in the 12 samples from wild Svalbard rock ptarmigans in summer (A) and the 17 samples from wild Svalbard rock ptarmigans in spring (B). Sample V6 had only a few reads assigned to other OTUs.

This sequencing run also included six samples from captive Svalbard rock ptarmigans, where most of the reads were assigned to *E. uekii* and only a few reads assigned to *Eimeria* sp. whilst no *E. rjupa* was detected in the samples from captive rock ptarmigan (Figure 13). In two of the samples taken from captive Svalbard rock ptarmigans at 7 days of age, we could find traces of vaccine strains such as *E. tenella* and *E. acervulina.* They had received a coccidiosis vaccine 5 days after hatching, and when they were re-sampled at 30 days of age, the vaccine strains were no longer present. The other sequencing run contained the 10 remaining samples from the captive Svalbard rock ptarmigans and all the reads mapping to *Eimeria* belonged to only 3 OTUs: all of them assigned to *E. uekii* with the same accession numbers as the top 2 OTUs from the previous sequencing run (Table 6).

Table 6 BLAST hits for representative sequences from the 3 OTUs from the second sequencing run in captive Svalbard rock ptarmigan. The table includes OTU ID, number of reads per OTU, accession numbers of matched reference sequences, species, percentage identity, e-values, and other relevant alignment metrics.

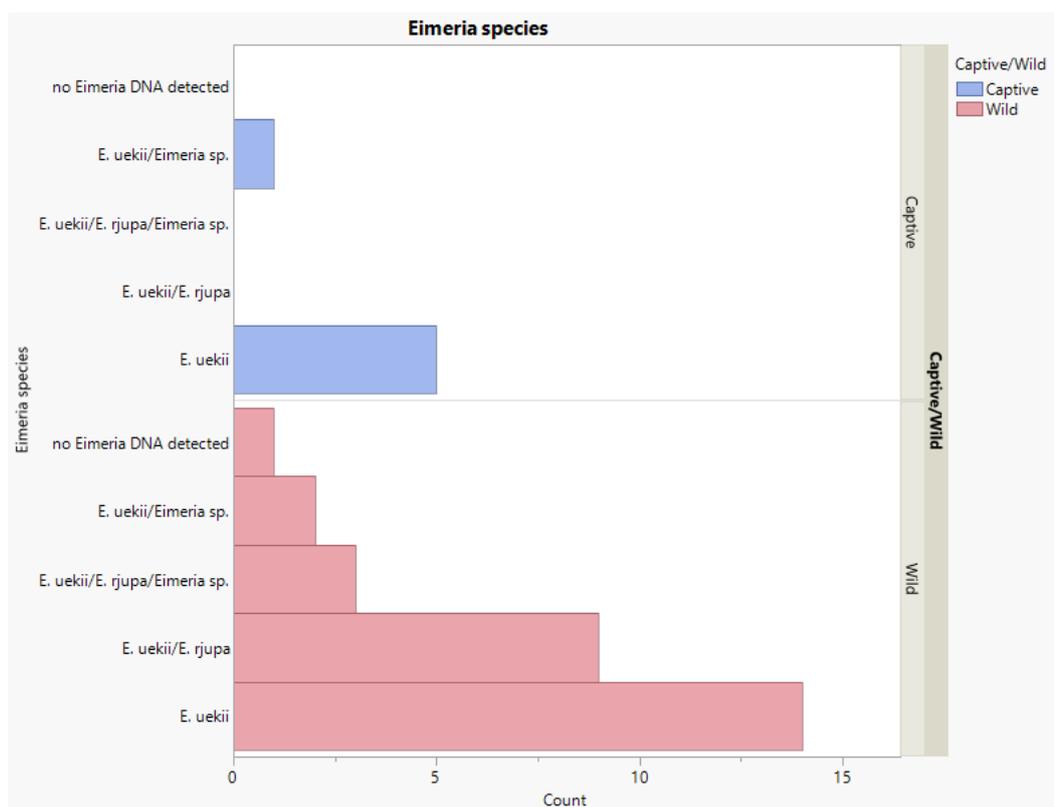| OTU_ID	Reads	% of total reads	Accession nr	Species	% identity	E-value	Bit score
OTU 1	2125814	69.5	LC380052	*Eimeria uekii*	99.8	0	763
OTU 2	873252	28.6	LC380046	*Eimeria uekii*	100	0	769
OTU 3	57454	1.9	LC380052	*Eimeria uekii*	97.1	0	702

Figure 13. Histogram showing the number of faecal samples from wild (n=6) and captive (n=29) Svalbard rock ptarmigan with single and mixed species *Eimeria* sp. infections, including *E. uekii, E. rjupa* and those in which the species was not determined after molecular analysis and sequencing, shown as *Eimeria sp.* One faecal sample tested negative for *Eimeria* DNA.

Closer investigation of the OPG by the dominant species identified in molecular analysis showed a difference in OPG between those infected with predominantly *E. rjupa* and *E. uekii* (Figure 14). Faecal samples dominated by *E. uekii* had higher mean (14751 OPG) and median (225 OPG) oocyst abundance than those infected with *E. rjupa* (Mean OPG 85; median OPG 0) but the significance level was not reached in non-parametric tests (p=0.06).
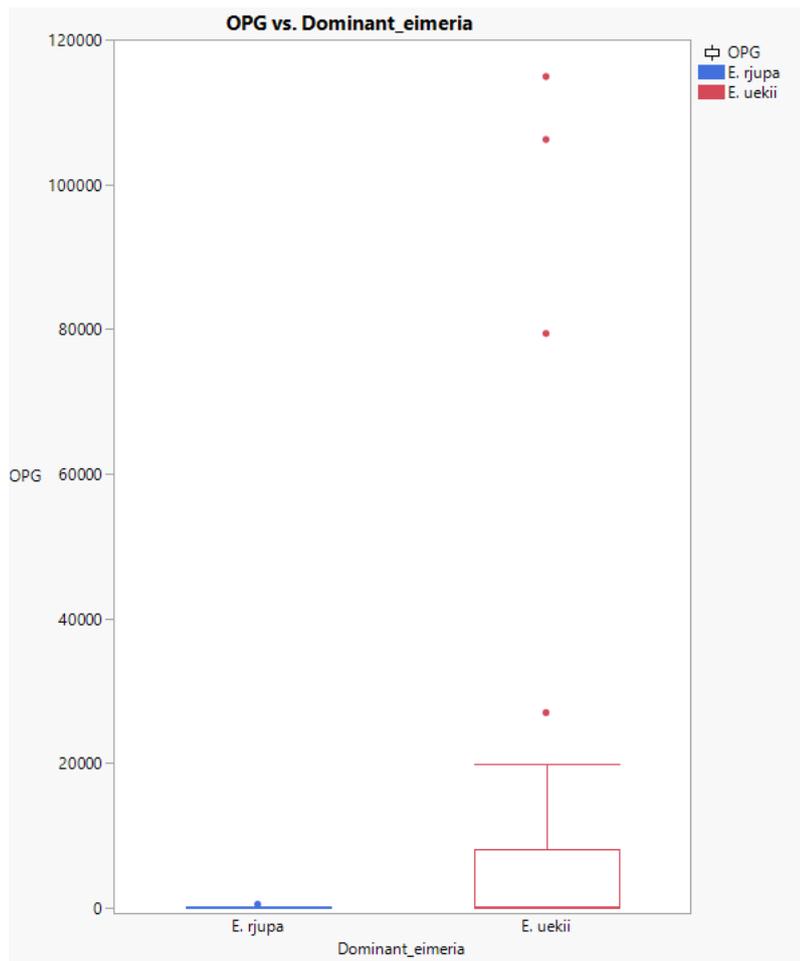
Figure 14. A box whisker plot with outliers showing the oocsts burden, OPG (oocysts per gram), plotted against the dominant *Eimeria* species found after sequencing in Svalbard rock ptarmigan.

Discussion

This study provides an assessment of coccidian infections in Svalbard rock ptarmigan, showing that *Eimeria* are widespread in both wild and captive birds and that wild individuals can reach oocyst burdens known to cause disease in captivity. The transmission and infection dynamics of parasites are influenced by different factors and processes like host density, prior immunity, the degree of environmental contamination, and climatic conditions may affect parasite development and survival outside the host. These density and contamination-dependent processes are particularly evident in captive rock ptarmigan populations, where outbreaks of coccidiosis have been documented (Matsubayashi et al., 2020; Norwegian Veterinary Institute unpublished data). The diet can also impact infection levels as some of the alpine plants eaten by rock ptarmigan have been shown to have potential anticoccidial activity (Haraguchi et al., 2024). It remains to be seen whether wild ptarmigan, with differing diets and densities, are equally susceptible to developing coccidiosis as those in captivity. Sporulation trials demonstrated strong temperature dependence, with rapid sporulation at ≥15 °C and evidence of cold and short-term freeze tolerance. Molecular analyses identified two species - *E. uekii* (dominant in both populations and associated with coccidiosis outbreaks in captive ptarmigan) and *E. rjupa* (present only in the wild). Together, these findings indicate that current and future Arctic warming may enhance environmental amplification of *Eimeria*, potentially affecting juvenile survival that may contribute to the declining juvenile proportion observed in long-term monitoring.

Even though oocysts were not detected in many of the samples, especially those from summer, **all samples, except one, had reads that were assigned to operational taxonomic units (OTUs) taxonomically classified as *Eimeria*.** It has been shown that oocyst counts correlate weakly with parasite intensity assessed through DNA quantification, as the DNA can be derived from other life-cycle stages than transmissive oocysts (Jarquín-Díaz et al., 2022). The OTUs were classified as *Eimeria* using the PR2 database for Eukaryotes, but without complete taxonomic species assignment. The database was able to identify the *Eimeria* species in the positive control (Paracox-5), which includes *Eimeria* species infecting chickens such as *E. acervulina* and *E. tenella* that are well studied and recognized as major pathogens in poultry production (Chapman, 2014; Shirley et al., 2005). *Eimeria* species infecting ptarmigan are less investigated and not included in the database. Therefore, further investigation of the OTUs had to be performed using NCBI blast (Altschul et al., 1990).

Two *Eimeria* species have previously been observed in the Svalbard rock ptarmigan. Initially they were first referred to as species A and B (Nielsen et al., 2024), and later identified as *E. uekii* and *E. rjupa*, respectively (Matsubayashi et al., 2024). BLAST results showed that the OTU sequences identified as *E. uekii* mapped to 18S rRNA sequences, accession number LC380052 and LC380046, submitted from a previous study of Matsubayashi et al. (2018a) of *Eimeria* in rock ptarmigan in Japan. The OTU sequence identified as *E. rjupa* mapped to the 18S rRNA sequence, accession number LC756964, submitted from a study of *Eimeria* in rock ptarmigan in Iceland, Svalbard and Japan (Matsubayashi et al. 2024). Matsubayashi et al. 2024 compared sequences of 18S rRNA and mitochondrial genes and detailed the morphology of sporulated oocysts of *E. uekii* and *E. raichoi* from Japan, *E. muta* and *E. rjupa* from Iceland, and the two undescribed *Eimeria* species A and species B from Norway (Svalbard). Matsubayashi et al.'s (2024) phylogenetic analysis suggested that *E. uekii*, *E. muta*, and *Eimeria* species A were identical, and that *E. rjupa* and Eimeria species B were identical. **We have therefore identified the OTUs in this study as either *E. uekii*, *E. rjupa*, or, if we were not able to confidently assign the OTU to a single *Eimeria* species, they were therefore classified as *Eimeria* sp.** The *Eimeria* sp. OTUs occurred at low abundance (≤2.8% of total reads) and could represent traces of an additional species that could not be resolved based on the 18S rRNA marker, alternatively they could represent sequences with high intraspecific variation, or potential sequencing/PCR artifacts.

One of the aims of the project was to assess which of the ptarmigan specific *Eimeria* species might cause disease in ptarmigan. It is interesting to note that *E. uekii* was the most prevalent species in the captive population and

potentially the cause of the coccidiosis outbreaks recorded in captive birds. This *Eimeria* species has also been linked to reduced body condition in Icelandic ptarmigan (Stenkewitz et al., 2016) and the same study found that *E. muta* (syn. *E. ueckii*) prevalence fluctuated, with a 1.5 year time lag, in parallel with the Icelandic rock ptarmigan population density, along with trends in reduced fecundity and increased host mortality related to increasing *E. muta* prevalence. Stenkewitz et al. (2016) concluded that this *Eimeria* species could destabilise ptarmigan population dynamics. However, in the cage in which the outbreak occurred one of the birds had *Eimeria* sp. DNA at very low levels (~1%) in addition to *E. uekii.* Interestingly the cage mate had milder clinical signs albeit a higher OPG and only *E. uekii* detected. Whilst we cannot comment on the pathogenicity of either species without carrying out further experimental infection trials, the evidence from this study, and from Stenkewitz et al. (2016) and Matsubayashi et al. (2023) strongly suggests that **E. uekii has pathogenic potential.** Recent experimental *E. uekii* and *E. raichoi* infections in captive Svalbard rock ptarmigan found that infective doses from 10 000 OPG resulted in clinical signs and reduced weight gains with coccidia predominantly detected in the small intestine (Matsubayashi et al., 2023). Evidence of acquired immunity was also demonstrated in this study using challenge infections. Whilst morphologically it appeared that there could be two species; one larger more oval and one smaller and round; molecular analysis indicated in some of the samples where both morphotypes were recorded nearly all the reads were identified as *E. uekii*. One explanation for this discrepancy could be oocysts floating at different angles in the fluid and appearing oval when floating horizontally and round when having a more vertical alignment. Molecular species confirmation is therefore crucial given known limitations to morphological species identification (Jitea (Sîrbu) et al., 2025).

The sporulation trials confirm that these Arctic adapted coccidia are capable of sporulation at a wide range of temperatures. The optimal range for sporulation appeared to be 15-25 °C whereby time to initial sporulation was less than a day and 50 % of oocysts in the sample were sporulated within approximately 2-4 days. A rapid build-up of infection in the environment requires rapid sporulation and a high sporulation prevalence. This was best seen in the samples that were incubated at 20 °C regardless of storage group (5 °C or -20 °C). Even at 5 °C, sporulation still occurred, although considerably more slowly: It took on average 10 days, with 50% of the oocysts sporulated at this time. However, sporulation success at 5 °C was influenced by storage history (see below). An earlier study found that rock ptarmigan *Eimeria* oocysts remained viable for up to 6 months at 4 °C (Matsubayashi et al., 2018b)**.** The sporulation percentages, in the wild Svalbard rock ptarmigan samples, were lower than in the captive Svalbard rock ptarmigan trial. One explanation of this could be related to length of environmental exposure prior to sampling. The captive Svalbard rock ptarmigan samples were fresher prior to storage and incubation whilst the wild Svalbard rock ptarmigan samples had been exposed to the elements for an unknown length of time prior to sampling and subsequent further storage. The researchers tried to obtain fresh samples based on the degree of desiccation. However, these differing degrees of freshness could perhaps account for some of the differences in sporulation success between the wild and captive groups.

Oocysts sporulated successfully after a week of freezing at -20 °C, indicating a degree of freeze tolerance. However, 9 months of continuous freezing exceeded their thermal tolerance and no sporulation occurred after these storage conditions. Freezing had temperature-dependent effects on subsequent sporulation success. Freezing prior to incubation at 5 °C had a detrimental effect on the sporulation success with less than 10 % of the oocysts sporulating compared to the samples that had been stored in the fridge when both were subsequently incubated at 5 °C. However, freezing did not appear to have such a negative effect with the samples incubated at 20 °C with no significant difference in sporulation percentages between the two storage groups.

The Arctic climate is warming at an unprecedented rate and as such these warmer temperatures can impact the transmission of climate sensitive infections like coccidian parasites (IPCC, 2018; Isaksen et al., 2022). **A warmer and wetter climate could speed up the transmission dynamics** with sporulation occurring within a day or two and higher sporulation success in fresher samples. This would suggest that there is a potential for a rapid build-up of infection pressure in years where temperatures rise above 15 °C and therefore a higher infection risk. We

did not explore what would happen at other storage temperatures and times, but this is worth future investigation. We currently know that these *Eimeria* spp. can survive freezing for at least a week and are capable of sporulation once exposed to warmer temperatures. We hypothesize that repeated freeze-thaw cycles would have a more detrimental effect on oocyst survival and sporulation so rain-on-snow events could limit the transmission of this parasite. However warmer and wetter spring temperatures would be ideal for transmission and could lead to disease outbreaks and/or reduced growth in young birds. Coccidian oocysts are also susceptible to inactivation by ultraviolet radiation (Baumann et al., 2025), and the continuous daylight, especially during Arctic summer, may therefore influence their ability to sporulate. In addition, warmer winters and earlier onset of spring could shift the timing of initial sporulation, impacting transmission risk. More work is needed to model the impact of the changing climate and seasonality on the expected sporulation rate and success of these Arctic coccidia and to explore in greater depth the impact of environmental temperature and light fluctuations to predict future disease risk.

One of the aims of the project was to investigate whether coccidia may be contributing to the negative trend of juvenile survival in wild Svalbard rock ptarmigan. Analysis of oocyst burdens in both wild and captive Svalbard rock ptarmigan shows that free-living birds can carry infections at levels comparable to those associated with disease outbreaks in captivity. Faecal samples from Bjørndalen, De Geerdalen, Adventdalen and Revneset all exceeded 15,000 OPG. In the captive population, prophylactic treatment (Dozuril 50 mg/ml: 2 ml/L in drinking water for seven days, followed by seven days without treatment and then repeated fortnightly throughout chick-rearing) was routinely administered due to the impact of coccidiosis on chick health and growth. For adult birds, treatment was initiated when monthly cage counts surpassed 15,000 OPG. Ideally, long-term surveillance of wild Svalbard rock ptarmigan should be undertaken to explore potential links between spring oocyst levels and the proportion of juveniles recorded in the autumn hunting bag. **While our current dataset supports the hypothesis that coccidiosis occurs in the wild at levels capable of causing disease, it remains insufficient to determine how much this parasite contributes to the apparent decline in juvenile proportions.**

Our findings indicate that coccidial burden (OPG) and prevalence could be used as ecological indicators for monitoring ptarmigan population health. The species, *E. uekii*, that was seen to be pathogenic in captivity is also the predominant species in the wild, underscoring the need to understand whether it has a possible effect on ptarmigan population levels. Incorporating long-term monitoring of coccidia prevalence and abundance into existing surveillance programs would help clarify whether fluctuations in parasite pressure correspond with changes in juvenile numbers in hunting statistics.

Figure 15. Male Svalbard rock ptarmigan in the wild. Photo: Eva Fuglei

References

Altschul, S.F., Gish, W., Miller, W., Myers, E.W., Lipman, D.J., 1990. Basic local alignment search tool. J. Mol. Biol. 215, 403–410. https://doi.org/10.1016/S0022-2836(05)80360-2

Baumann, A.A., Myers, A.K., Khajeh-Kazerooni, N., Wise-Mitchell, A., Rosenthal, B.M., Jenkins, M.C., O'Brien, C., Fuller, L., Tremaine, S., Morgan, M., Lenaghan, S.C., 2025. UV radiation at 222, 254, and 282 nm inhibits sporulation and suppresses infectivity of *Eimeria acervulina* oocysts. Microbiol. Spectr. 13, e02439-24. https://doi.org/10.1128/spectrum.02439-24

Berto, B.P., Lopes, C.W.G., 2020. Coccidia of Wild Birds as Ecological Biomarkers: Some Approaches on Parasite-Host-Environment Interaction. J. Parasitol. 106, 707–713. https://doi.org/10.1645/19-148

Brinkmann, A., 1927. Coccidiosen hos lirypen. Bergens Mus. Aarb. 1926 Naturvidenskabelig Række 9, 75.

Chapman, H.D., 2014. Milestones in avian coccidiosis research: A review. Poult. Sci. 93, 501–511. https://doi.org/10.3382/ps.2013-03634

COAT, 2024. Rype Svalbard [WWW Document]. COAT. URL https://coat.no/no/Rype/Svalbard

Davidson, R.K., Handeland, K., Kapel, C.M.O., 2008. High tolerance to repeated cycles of freezing and thawing in different *Trichinella nativa* isolates. Parasitol. Res. 103, 1005–1010. https://doi.org/10.1007/s00436-008-1079-0

de Muinck, E.J., Trosvik, P., Gilfillan, G.D., Hov, J.R., Sundaram, A.Y.M., 2017. A novel ultra high-throughput 16S rRNA gene amplicon sequencing library preparation method for the Illumina HiSeq platform. Microbiome 5, 68. https://doi.org/10.1186/s40168-017-0279-1

Fuglei, E., Henden, J.-A., Callahan, C.T., Gilg, O., Hansen, J., Ims, R.A., Isaev, A.P., Lang, J., McIntyre, C.L., Merizon, R.A., Mineev, O.Y., Mineev, Y.N., Mossop, D., Nielsen, O.K., Nilsen, E.B., Pedersen, Å.Ø., Schmidt, N.M., Sittler, B., Willebrand, M.H., Martin, K., 2020. Circumpolar status of Arctic ptarmigan: Population dynamics and trends. Ambio 49, 749–761. https://doi.org/10.1007/s13280-019-01191-0

Fuglei, E., Holmgaard, S., Stien, J., Tombre, I., Pederson, Å., 2019. Svalbardrypenes jakstatistikk. Sluttrapport 16/54 til Svalbards Miljøvernfond (No. Kortrapport nr. 051). Norsk Polarinstitutt.

Fuglei, E., Paulsen, I., Pederson, Å., 2023. Høsttelling av svalbardrype. Sluttrapport til Svalbards miljøvernfond. (No. Rapport 154). Norsk Polarinstitutt.

Graat, E.A., Henken, A.M., Ploeger, H.W., Noordhuizen, J.P., Vertommen, M.H., 1994. Rate and course of sporulation of oocysts of *Eimeria acervulina* under different environmental conditions. Parasitology 108 (Pt 5), 497–502. https://doi.org/10.1017/s0031182000077350

Guillou, L., Bachar, D., Audic, S., Bass, D., Berney, C., Bittner, L., Boutte, C., Burgaud, G., de Vargas, C., Decelle, J., Del Campo, J., Dolan, J.R., Dunthorn, M., Edvardsen, B., Holzmann, M., Kooistra, W.H.C.F., Lara, E., Le Bescot, N., Logares, R., Mahé, F., Massana, R., Montresor, M., Morard, R., Not, F., Pawlowski, J., Probert, I., Sauvadet, A.-L., Siano, R., Stoeck, T., Vaulot, D., Zimmermann, P., Christen, R., 2013. The Protist Ribosomal Reference database (PR2): a catalog of unicellular eukaryote small sub-unit rRNA sequences with curated taxonomy. Nucleic Acids Res 41, D597-604. https://doi.org/10.1093/nar/gks1160

Haraguchi, A., Nagasawa, J., Kuramochi, K., Tsuchida, S., Kobayashi, A., Hatabu, T., Sasai, K., Ikadai, H., Ushida, K., Matsubayashi, M., 2024. Anticoccidial activity of the secondary metabolites in alpine plants frequently ingested by wild Japanese rock ptarmigans. Int. J. Parasitol. Parasites Wildl. 25, 100967. https://doi.org/10.1016/j.ijppaw.2024.100967

Hinsu, A.T., Thakkar, J.R., Koringa, P.G., Vrba, V., Jakhesara, S.J., Psifidi, A., Guitian, J., Tomley, F.M., Rank, D.N., Raman, M., Joshi, C.G., Blake, D.P., 2018. Illumina Next Generation Sequencing for the Analysis of *Eimeria* Populations in Commercial Broilers and Indigenous Chickens. Front. Vet. Sci. Volume 5-2018.

IPCC, 2018. Global Warming of 1.5 ºC. An IPCC Special Report on the impacts of global warming of 1.5°C above pre-industrial levels and related global greenhouse gas emission pathways, in the context of strengthening the global response to the threat of climate change, sustainable development, and efforts to eradicate poverty (IPCC Special Report). Cambridge University Press, Cambridge, UK. https://doi.org/10.1017/9781009157940

Isaksen, K., Nordli, Ø., Ivanov, B., Køltzow, M.A.Ø., Aaboe, S., Gjelten, H.M., Mezghani, A., Eastwood, S., Førland, E., Benestad, R.E., Hanssen-Bauer, I., Brækkan, R., Sviashchennikov, P., Demin, V., Revina, A., Karandasheva, T., 2022. Exceptional warming over the Barents area. Sci. Rep. 12, 9371. https://doi.org/10.1038/s41598-022-13568-5

Jarquín-Díaz, V.H., Balard, A., Ferreira, S.C.M., Mittné, V., Murata, J.M., Heitlinger, E., 2022. DNA-based quantification and counting of transmission stages provides different but complementary parasite load estimates: an example from rodent coccidia (*Eimeria*). Parasit. Vectors 15, 45. https://doi.org/10.1186/s13071-021-05119-0

Jitea (Sîrbu), B.A., Morariu, S., Imre, M., Florea, T., Sîrbu, C.B., Luca, I., Dumitru, S., Dărăbuş, G., 2025. Microscopic and Molecular Identification of *Eimeria* Species in Domestic Rabbits (Oryctolagus cuniculus) in Romania. Animals 15, 1109. https://doi.org/10.3390/ani15081109

John-André Henden, Rolf Anker Ims, Eva Fuglei, Åshild Ønvik Pedersen, 2017. Changed Arctic-alpine food web interactions under rapid climate warming: implication for ptarmigan research. Wildl. Biol. 2017. https://doi.org/10.2981/wlb.00240

Lassen, B., Seppä-Lassila, L., 2014. Recovery and sporulation of bovine *Eimeria* oocysts after exposure to sub-zero temperature. Vet. Ir Zootech. Vet Med Zoot 66, 35–39.

Marolla, F., Henden, J.-A., Fuglei, E., Pedersen, Å.Ø., Itkin, M., Ims, R.A., 2021. Iterative model predictions for wildlife populations impacted by rapid climate change. Glob. Change Biol. 27, 1547–1559. https://doi.org/10.1111/gcb.15518

Matsubayashi, M., Kinoshita, M., Kobayashi, A., Tsuchida, S., Shibahara, T., Hasegawa, M., Nakamura, H., Sasai, K., Ushida, K., 2020. Parasitic development in intestines and oocyst shedding patterns for infection by *Eimeria uekii* and *Eimeria raichoi* in

Japanese rock ptarmigans, *Lagopus muta japonica*, protected by cages in the Southern Japanese Alps. Artic. Spec. Issue 'Parasites Extreme Environ. Pp 250 - 325 12, 19–24. https://doi.org/10.1016/j.ijppaw.2020.04.002

Matsubayashi, M., Kinoshita, M., Tsuchida, S., Kobayashi, A., Tamura, N., Shibahara, T., Kido, Y., Kaneko, A., Sasai, K., Ushida, K., 2023. Experimental evaluation of pathogenicity and acquired immunity of Eimeria species, *E. uekii* and *E. raichoi,* infecting Japanese rock ptarmigans in a subspecies of the birds. Int. J. Parasitol. Parasites Wildl. 22, 167–174. https://doi.org/10.1016/j.ijppaw.2023.09.005

Matsubayashi, M., Tsuchida, S., Kobayashi, A., Shibahara, T., Nakamura, H., Murata, K., Ushida, K., 2018a. Molecular identification of two *Eimeria* species, *E. uekii* and *E. raichoi* as type B, in wild Japanese rock ptarmigans, *Lagopus muta japonica*. Int. J. Parasitol. Parasites Wildl. 7, 243–250. https://doi.org/10.1016/j.ijppaw.2018.06.005

Matsubayashi, M., Tsuchida, S., Shibahara, T., Ushida, K., Fuglei, E., Pedersen, Å.Ø., Nielsen, Ó.K., Duszynski, D.W., Skírnisson, K., 2024. Comparative molecular analyses of *Eimeria* Schneider (Apicomplexa: Eimeriidae) species from rock ptarmigan in Iceland, Svalbard-Norway, and Japan. Syst. Parasitol. 101, 31. https://doi.org/10.1007/s11230-024-10159-y

Matsubayashi, M., Tsuchida, S., Ushida, K., Murata, K., 2018b. Surveillance of *Eimeria* species in wild Japanese rock ptarmigans, *Lagopus muta japonica*, and insight into parasitic seasonal life cycle at timberline regions of the Japanese Alps. Int. J. Parasitol. Parasites Wildl. 7, 134–140. https://doi.org/10.1016/j.ijppaw.2018.03.004

MOSJ, 2023. Svalbard rock ptarmigan [WWW Document]. Environ. Monit. Svalbard Jan Mayen. URL https://mosj.no/en/indikator/fauna/terrestrial-fauna/svalbard-rock-ptarmigan/

Nielsen, Ó.K., Fuglei, E., Pedersen, Å.Ø., Skírnisson, K., 2024. Svalbard rock ptarmigan: a first glimpse into parasite infections. Polar Res. 43. https://doi.org/10.33265/polar.v43.9506

Pedersen, Å.Ø., Bårdsen, B.-J., Yoccoz, N.G., Lecomte, N., Fuglei, E., 2012. Monitoring Svalbard rock ptarmigan: Distance sampling and occupancy modeling. J. Wildl. Manag. 76, 308–316. https://doi.org/10.1002/jwmg.276

Shirley, M.W., Smith, A.L., Tomley, F.M., 2005. The Biology of Avian *Eimeria* with an Emphasis on their Control by Vaccination, in: Baker, J.R., Muller, R., Rollinson, D. (Eds.), Advances in Parasitology. Academic Press, pp. 285–330. https://doi.org/10.1016/S0065-308X(05)60005-X

Soininen, E.M., Fuglei, E., Pedersen, Å.Ø., 2016. Complementary use of density estimates and hunting statistics: different sides of the same story? Eur. J. Wildl. Res. 62, 151–160. https://doi.org/10.1007/s10344-016-0987-z

Stenkewitz, K., Nilesen, Ó., Skírnisson, K., Stefánsson, G., 2016. Host-Parasite Interactions and Population Dynamics of Rock Ptarmigan. PLOS One 11, e0165293. https://doi.org/10.1371/journal.pone.0165293

Straub, D., Blackwell, N., Langarica-Fuentes, A., Peltzer, A., Nahnsen, S., Kleindienst, S., 2020. Interpretations of Environmental Microbial Community Studies Are Biased by the Selected 16S rRNA (Gene) Amplicon Sequencing Pipeline. Front. Microbiol. Volume 11-2020.

Taylor, M., Coop, R., Wall, R., 2016. Veterinary Parasitology, 4th Edition. ed. Wiley Blackwell.

Waldenstedt, L., Elwinger, K., Lundén, A., Thebo, P., Uggla, A., 2001. Sporulation of *Eimeria maxima* oocysts in litter with different moisture contents. Poult. Sci. 80, 1412–1415. https://doi.org/10.1093/ps/80.10.1412

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