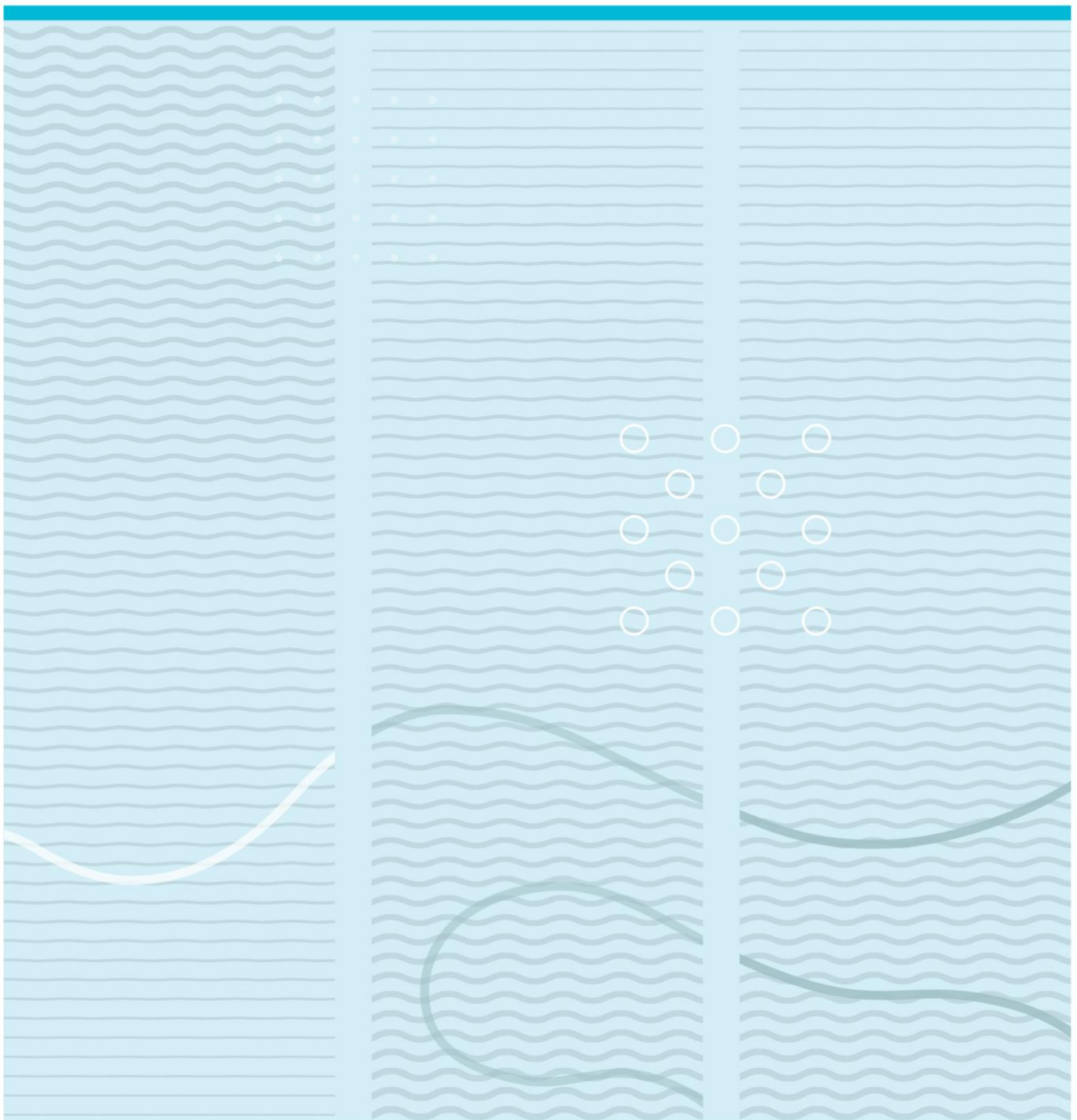Charlotte Holmstad Arnesen

Canines as biodetectors for conservational work: can they discriminate Rock ptarmigan from Willow ptarmigan?

This thesis is worth 60 study points

Abstract

The main goals for conservation biology is to document the full range of biological diversity and human impact, along with developing plans, approaches and measures to prevent extinction of species. Alpine and arctic bird populations have shown an unmistakable decrease in the last three decades, whereas the Rock ptarmigan (*Lagopus muta*) and Willow ptarmigan (*L. lagopus*) appear to have experienced one of the most severe decline, which have placed the respective species on the Norwegian "*red list for threatened species*" and in addition the Rock ptarmigan on the European red list in the category: "*Near threatened*". In this study, the potential use of four privately owned dogs (*Canis lupus familiaris*) as a non-invasive conservational tool to determine the presence or absence of Rock ptarmigan through sniffing out fecal pellets (N=210) collected in 32 different areas in Norway and Sweden in laboratory conditions was investigated. I hypothesized that dogs could recognize fecal pellets from the Rock ptarmigan and discriminate it from other bird species in the *Tetraonidae* family (Black grouse (*Tetrao Tetrix*), Western capercaillie (*T. urogallus)* and Willow ptarmigan). I predicted that dogs would do a trained final response and lay down in front of the fecal pellets from the Rock ptarmigan if present and return to handler when absent. I showed that dogs can detect odor differences between the two avian species with an average accuracy of 66%, sensitivity of 67% and specificity of 65%. The result revealed a considerable range between the poorest and strongest performing dog in sensitivity (33% and 94%), specificity (44% and 89%) and accuracy (61% and 81%,). Looking at the result through a system consisting of four dogs, where $\geq$ three dogs were required to perform an equal response on an arbitrarily sample for it to be considered a valid evaluation, we were able to reduce the cost of species identification through DNA-analysis by 55.6%. The result in this study demonstrates that dogs can be trained to recognize fecal pellets from the Rock ptarmigan and discriminate it from other similar species. The system approach can serve as an additional tool to fecal analysis providing management and researchers with information about the endangered species.

Keywords: Conservation, discrimination, dogs, fecal pellets, *Lagopus lagopus*, *Lagopus muta*, non-invasive method.

Contents

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1. Introduction

The main goals for conservation biology is to document the full range of biological diversity and human impact, along with developing of plans, approaches and measures to prevent extinction of species (Primack 2014). To initiate any kind of conservational or managing action, fundamental information about the species and its population is required (Smallwood and Schonewald 1998). Information that have proven to be quite challenging to comprehend, as the threatened species often appear to be rare, vulnerable, occupying habitats that serves difficulties for humans to monitor and survey, and sometimes being both small and cryptic (Piggott and Taylor 2003, Primack 2014).

Alpine and artic bird populations have shown an unmistakable decrease in the last three decades, whereas the Rock ptarmigan (*Lagopus muta)* and the Willow ptarmigan (*L. lagopus)* appear to have experienced one of the most severe decline (Byrkjedal and Kålås 2012, Lehikoinen et al. 2014), which have placed the respective species on the "*Red list for threatened species*" in Norway (Henriksen and Hilmo 2015) and in addition the Rock ptarmigan on the European red list (BirdLife 2015) in the category: "*Near threatened"*. The Rock ptarmigan and the Willow ptarmigan are existing sympatric in a large part of their distributional range (Storch 2000, Watson and Moss 2008, Quintela et al. 2010). They share a substantial amount of their life story, several morphological traits, as well as cultural traditions for humans as game birds (Watson and Moss 2008, Nilsen et al. 2012). Usually the Rock ptarmigan occupies habitats in higher elevation than the Willow ptarmigan (Storch 2000), but in arctic areas the Rock ptarmigan are found breeding at sea level (Watson and Moss 2008). Potential threats as both climatic changes (Thomas and Lennon 1999, Thomas 2010) and disturbance from humans (Storch 2000, Viñuela and Arroyo 2002, Storch 2007) are expected to further increase the possibility for habitat overlap by extending the Willow ptarmigans habitat upwards and simultaneously decreases the Rock ptarmigans habitat (Quintela et al. 2010).

Relative to the Willow ptarmigan, there is a general lack of information about the Rock ptarmigan, especially when it comes to estimation of the population. Several possible threats are identified and stated to have influenced the decline. Climate change in relation to e.g. uphill- and poleward movement creating fragmented habitats and isolated populations (Thomas and Lennon 1999, Thomas 2010), food sources (Jepsen et

al. 2008, Brommer and Møller 2010, Thomas 2010), and breeding challenges as for example site and time selection (Martin and Wiebe 2004, Wilson and Martin 2010). Increased predator pressure which relates to e.g. rodents cycles (Kausrud et al. 2008) and alternative preys of predators (Reif et al. 2001, Tornberg et al. 2011). Human disturbances in relation to recreation as e.g. building of ski resorts, thus introducing new predators that is attracted to human waste (e.g. crows (*Corvus cornix*) and red fox (*Vulpes vulpes*)) (Watson and Moss 2004), increased adult mortality in relation to collisions in power lines and reindeer (*Rangifer tarandus)* fencing (Bevanger and Brøseth 2000, Watson and Moss 2004) together with harvesting (Viñuela and Arroyo 2002, Lehikoinen et al. 2014).

These complexed and cooperative threats have resulted in small isolated populations for the Rock ptarmigan, where one of the main reason is habitat fragmentations (Storch 2000, Lehikoinen et al. 2014). The fragmentation is creating barriers to e.g. immigration, resulting in reduced number of new individuals in the respective populations, hence a reduced gene flow (Bhattacharya et al. 2003, Banks et al. 2005, Primack 2014).While the adult males tend to stay in their habitat for territorial defense, females are more likely to show emigrational behavior leaving their birth place (Greenwood 1980), a behavior strengthened and often seen in fragmented habitats and isolated bird populations (Dale 2001, Steifetten and Dale 2006, Donald 2007). Female migration have also been shown to result in higher mortality compared to males, by e.g. crossing unknown territory and unknown food supply (Dale 2001, Steifetten and Dale 2006). These patterns are helping an emerging abundance of males, and losses of available females for mating, resulting in a skew operational sex ratio (OSR), a fundamental demographic tool in a population that has to be considered in wildlife managing (Greenwood 1980, Walters et al. 1999, Steifetten and Dale 2006, Donald 2007).

Population monitoring of the Rock ptarmigan tend to be challenging, but in a few appropriate areas, capture-recapture methods (Amstrup et al. 2005) are used, or plot-based (predetermined random plots, sampling calling activities in spring using "occupancy-models" and "distance-models" for analysis) or line-based (walking randomly chosen line transect, sampling observed individuals using "occupancy-models" and/or "distance-models" for analysis) surveys (Buckland et al. 2005,

MacKenzie et al. 2017). The two last mentioned methods are successfully used with e.g. different kinds of songbirds and Willow ptarmigan (Buckland et al. 2005, Buckland 2006). For the Rock ptarmigan, plot-based surveys is the methodology most frequently used (Marty and Mossoll-Torres 2012, Nilsen et al. 2012), where observed individuals and observed calling activity (territorial males) are documented and analyzed with the assumption that the OSR is 1:1 (Carvalho et al. 1998, Nilsen et al. 2012).

Currently population monitoring methods have proven to be both time consuming and slightly ineffective (Beckoff and Jamieson 1996, Greenwood 1996, Steifetten and Dale 2006, Nilsen et al. 2012). To follow a line transect or walk to a certain plot that is randomly selected without consider the terrain in alpine and arctic mountain areas tend to be quite challenging (Marty and Mossoll-Torres 2012, Nilsen et al. 2012). Animals are often captured with some kind of bate or require the animal to implement a certain behavior, like moving pass a specific position which present the researcher with animals that is not representative for the rest of population (Greenwood 1996) or by assuming that the OSR is 1:1, an assumption that often provides an incorrect reality of the respective population, especially in small and isolated populations (Dale 2001, Steifetten and Dale 2006, Donald 2007). Manufacturing conservation plans and measures on the basis of that information can dismantle any action initiated (Steifetten and Dale 2006, Donald 2007).

Collecting fecal matter from animals is an important non-invasive tool that serves the researcher a lot of information about the species and its population, not only in field, but also in the laboratory e.g. abundance, habitat use, movement, sex ratio, age, diets, reproductive productivity and hormones- and stress-levels (Putman 1984, Wasser et al. 1996, Kohn and Wayne 1997, Berger et al. 1999). All this information provided by a by-product from animals and the requirement of any visual- and physical contact is redundant, thus no harm or risk is applied to the endangered specimen. Several studies have collected fecal matter from animals to get more information about threatened species like e.g. estimating population size and trends of Black bears (*Ursus arctos)* in Sweden (Kindberg et al. 2011) and population monitoring of Snow leopards (*Pantera unicia*) in north-western India, central China and southern Mongolia (Janečka et al. 2008).

A possible non-invasive tool for fecal detection and species discrimination could be the use of dogs (*Canis lupus familiaris*), a macrosomatic animal with a highly sensitive sense of smell, whom have been used as field assistants for humans in conservation, research and management for many years (Woollett (Smith) et al. 2013, Rosell 2018). Long et al. (2007) showed that dogs achieved an increased detection rate of Bobcats (*Lynx rufus*) by ten times compared to automatic cameras, hair snares and scent stations. Wasser et al. (2012) compared fecal detection dogs to occupancy-methods by eliciting vocalization responses of the Northern spotted (*Strix occidentalis*) and Barred (*S. varia*) owls, and the result showed that dogs had a significantly higher detection rate than the vocalization surveys. Cristescu et al. (2015) trained dogs to find fecal matter from Koala bears (*Phascolarctos cinereus*) which resulted in detection dogs to be 19 times more efficient than humans and performed with a detection rate of 100%. Orkin et al. (2016) trained dogs in collaboration with international primatologists and the Chinese ministry to detect fecal matter from the Indochinese Gray Langur (*Trachypithecus crepusculus*), Western Black Crested Gibbon (*Nomascus concolor*) and the Macaque (*Macaca sp.*), achieving an accuracy of 92%, which significantly outperformed human-only searching teams.

Studies have also shown that dogs can be highly sensitive in species discrimination with close related species using fecal matter as well. Smith et al. (2003) trained detection dogs to recognize San Joaquin kit foxes (*V. macrotis mutica*) and discriminate it from other sympatric fox species and coyotes (*Canis latrans*), all dog-humans teams outperformed human-only teams in field search. Hurt et al. (2000) trained dogs to distinguish black bear from grizzly bears (*U. americanus*) with an detection accuracy ranging from 65-85%. Rosell et al. (Submitted) trained dogs in laboratory conditions to discriminate between the two beaver (*Castor spp.*) species, Eurasian beaver (*Castor fiber*) and the North American beaver (*C. canadensis*) via castoreum (fluid that primarily contains concentrated urine, one of two fluids that beavers use for scent marking (Rosell and Bergan 1998, Rosell and Sundsdal 2001)) collected from dead beavers. The study also reveals that the dogs were successfully able to discriminate between fresh scent mark samples collected from living beaver colonies, when they only had been trained using 15 years old castoreum samples.

In this study, the potential use of dogs as a non-invasive conservational tool to determine the presence or absence of Rock ptarmigan through sniffing out fecal pellets collected in 30 different mountain areas in Norway and Sweden was investigated. I hypothesized that the dogs could recognize the Rock ptarmigan and discriminate it from the closely related Willow ptarmigan. I predicted that the dogs would do a trained final response (TFR) and lay down in front of the fecal pellets from Rock ptarmigan if present and return to handler when absent.

2. Methods

2.1. Dogs

Five privately owned dogs with a mean age of 7.4 years (SD ± 4.07) and a basic level of obedience were used in training (Tab. 1). Two women handled the dogs, and they were not professional dog trainers, but both with a scientific background. One handler (B) and four dogs had been used in earlier scent detection work (Tab. 1) (Rosell et al. Submitted).

Table 1. The dogs used, their sex (F = female, M = male), age (year), breed, handler, ownership with their handler, scent detection experience and total number of training sessions.

Dog	Sex	Age	Breed	Handler	Ownership	Scent detection experience	Training sessions
Akira	F	1.5	Grosspitz	A	Yes	No	76
Bailey	M	5	Nova Scotia duck tolling retriever	B	Yes	Yes	79
Chilli	F	9	Border collie	A	No	Yes	83
Shib	F	11.5	Border collie	B	No	Yes	83
Tapas	M	9	Border collie	A	No	Yes	27

2.2. Scent donors

Fecal pellets from 85 Rock ptarmigans, 85 Willow ptarmigans, 20 Western capercaillie (*Tetrao urogallus*) and 20 Black grouse (*T. tetrix*) were collected from spring 2015 to winter 2016. Forty-three samples of Rock ptarmigan, 43 samples Willow ptarmigan, 20 samples of Western capercaillie and 20 samples of Black grouse were used in training, and the remaining used in the final experiments (See below). The Rock ptarmigan and Willow ptarmigan samples were collected in 30 different mountain areas in Norway and Sweden in context with another study for DNA analysis of the respective species, thus identified on species and individual levels (Appendix 1 (Costanzi et al. 2018, Ring et al. In prep)). Varying quality (different age and degree of degradation through outdoor aging) of fecal pellets were used, but all pellets were morphological intact and only used if they were able to be identified through DNA analysis. Training dogs with fecal matter with varying quality will provide them with an extensive scent impression of the species (Wasser et al. 2004, Mackay et al. 2008).

When the Rock ptarmigan and Willow ptarmigan samples were collected in field, they were directly placed in plastic jars with a screw cap (Nalgene, 15 x 38 mm or 30 x 43 mm, Thermo Scientific ™, Norway) with GPS coordinates, site and ID number. When sampling was completed, the content of the jars was taken out and placed on individual paper towels for seven days to dry. Time from sampling to the drying process was completed could take from five hours and up to two weeks. After the pellets were dry, new jars were filled with silica gel, and a precision wipe (Kimberly-Clark ™, Professional 05511, Canada) was added to separate the gel from the pellets. Samples was stored in a fridge at 2-4°C and the storing time varied from time sampled (spring 2015 to fall 2016) to the time they were added to this study sample collection in October 2016. An average of two periods with open lids and DNA-analysis were carried out per sample.

By collecting samples from a large number of areas, the dogs will be provided with fecal samples containing several different odor stimuli from the same species, thus a generalized odor impression is contrived and the chances that the dogs will pick up individual-specific odor stimulus from a small number of birds as e.g. diet and sex decreases (Wasser et al. 2004, Willis et al. 2004, Furton et al. 2010). Training dogs on a large sample size, will further decrease the chances of them to recognize and remember individual samples, rather than the general scent impression of the species (Johnen et al. 2013, Elliker et al. 2014). Fecal pellets that have been dried and stored in room temperature and a fridge for that considerable amount of time have probably experienced a great loss of volatile compounds and decreased quality (Smith et al. 2003, Wasser et al. 2004, Rosell et al. Submitted)

The fecal pellets got weighted using an analytical balanced weight (AND Electronic balance FA-200, AC adapter DC 12V 0.3A, China) and placed in glass vials with Teflon lids (57 x 27.5 mm, Qorpak®, Pennsylvania, USA). The mean weight of the samples was 0.7 grams (SD ± 0.078 grams). Every sample was handled with a new pair of disposable gloves and tweezer sterilized with an open flame and laboratory ethanol (96%) to avoid cross contamination. They were stored in a freezer at – 20ºC until they were used in either training or experiments (see below).

Sampling of fecal pellets from Black grouse and Western capercaillie was independent from the sampling of the ptarmigans and collected between October and December 2016.

They were collected in four different lowland, forest areas in Telemark and Buskerud counties, Norway. There was no considerable chance of habitat overlap with either the Rock ptarmigan or the Willow ptarmigan as they were not reported in those areas. The fecal pellets collected was placed in individual paper bags with ID and site number. After arriving to the laboratory, they were morphological identified and dried for seven to ten days. After the fecal pellets was dried, they were placed in glass vials with teflon lids and immediately stored in the freezer at -20ºC.

2.3. Dog training

Training took place at the dog laboratory at the University of South-eastern Norway, Bø in Telemark county, and was carried out from the 25th of October 2016 to 5th of January 2018. The laboratory consisted of two separate rooms, a training room and an observer room. In the training room it was installed two cameras (Sony Handycam DCR-SR 35, UK) that was connected to a monitor and screen in the observer room allowing the experimenter to directly watch the dog-handler team without interrupting. Each dog was trained periodically between 1-3 sessions a week (only one session per day). Duration of a training session and whether the dogs trained alone or in a joint session varied, but one dog trained for approximately 15-20 minutes per session.

All the training was exclusively based on positive reinforcement through operant conditioning by using of a clicker-sound as the second reinforcer and receiving of each dog preferable reward (food treats, praise and/or toy) (Furton et al. 2010, Deldalle and Gaunet 2014, Chiandetti et al. 2016). All the dogs were familiar with the sound of a clicker and receiving of a reward.

2.3.1. Training procedures

Training was split into three phases: 1) adaption to laboratory and initial training on the table platform, 2) discrimination training, and 3) adaption to a yes/no training regime. Each phase contained smaller goals, in which they had to reach by showing repeatability and reliability before moving on to the next (Furton et al. 2010, Fischer-Tenhagen et al. 2011, Jezierski 2016).

A table platform adapted in Hällefors, Sweden, by the Hundcampus training center was used in all training phases (Fig. 1A, see also (Fischer-Tenhagen et al. 2011)). The platform is made of stainless-steel and plexi glass and consists of a plank with seven holes, two plexi glass together with a stainless-steel sample cover and a sloped wall defining four out of the seven holes, slightly elevated from the original stainless-steel plank (Fig. 1A). On the back of the platform, a handle is connected to one of the holes, this enables the dog handler to move the plank, thus the samples, back and forth, and is also implying the location of the target odor. To emphasis that the dogs learned to both recognize and discriminate Rock ptarmigan from other odors, a collection of control samples were created (McCulloch et al. 2006, Elliker et al. 2014). Fecal pellets from birds in the same family (*Tetraonidae)* and the same genus, (*Lagopus*) were used, and in addition other control samples as: empty glass vials, empty glass vials with identical marking to those of the scat samples, sterilized plastic cups, plastic cup with a jar housing silica gel and a piece of paper, plastic cups with a disposable glove and a blank (nothing). These control samples were objects used in preparation of the samples or during training as glass vials with and without identical marking as to those of the fecal pellets, sterilized plastic cups used for housing the glass vials with fecal pellets inside the table platform, silica gel in plastic jar which was added to the fecal pellets during drying period and disposable glove used during preparation. The target odor was always within the defined four holes of the table platform. Thus, the dog was presented with four options in every lineup, i.e. the target odor, Rock ptarmigan, and three other control samples. The control samples were randomly placed within the six remaining holes. In addition to the table platform, a two-holed floor platform was used as an intermediate step to the final training regime: a yes/no training regime, where the dogs were to make a yes-decision, lay down in front of the platform when target was present, or a no-decision, return to handler when target was absent using a single-holed floor platform (Fig. 1B and 1C) (Frederick et al. 2011, Gadbois and Reeve 2016, Fischer-Tenhagen et al. 2017).

A) Table platform

- Blank
- Black grouse
- Willow ptarmigan
- Target: Rock ptarmigan
- Silica gel
- Western capercaillie

B) Two-holed floor platform

- Rock ptarmigan
- Willow ptarmigan/ Blank

C) One-holed floor platform

- Rock ptarmigan/ Willow ptarmigan/ Blank
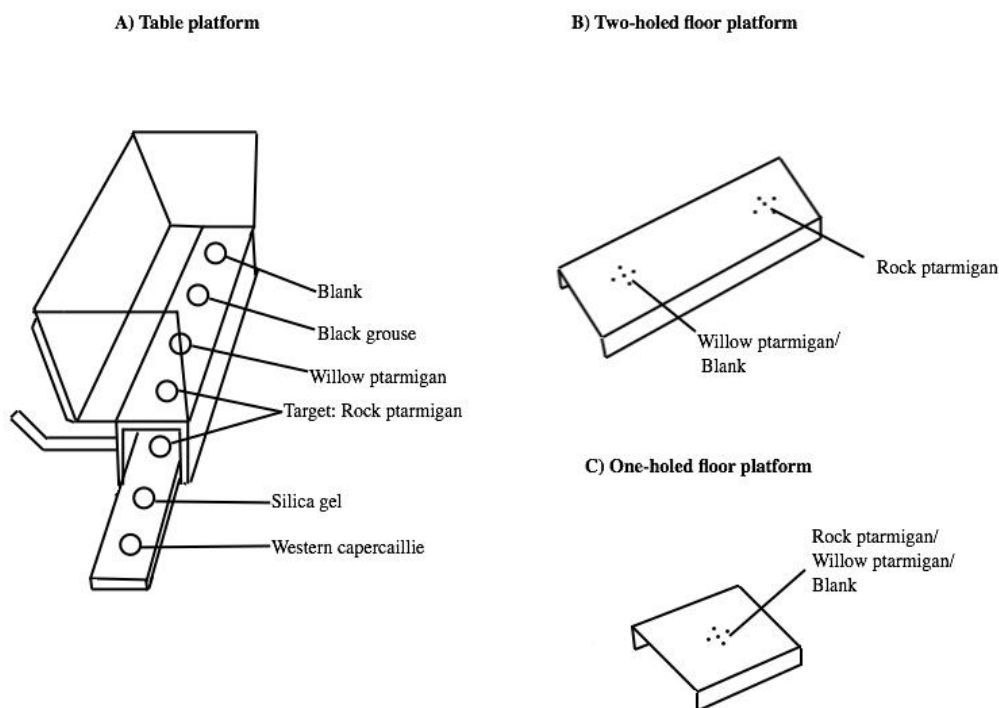
Figure 1. The platforms used in training and experiments: (A) table platform presenting the dog with four out of seven options, the handle is connected to the target sample which enables the handler to move the plank, thus the samples back and forth, (B) two-holed platform used as an intermediate step in the adaption from floor platform to the one-holed platform in phase three, presenting the dog with two options, (C) one-holed platform used in phase three and experiments, presenting the dog with one option, the dog are required to make a "yes" or a "no" decision. Note that all training sessions involved a random sample layout, and platform (A) could contain additional control samples as well.

To avoid (cross) contamination disposable gloves were worn at all times when interacting with the samples, and samples in the table platform were placed in sterilized plastic cups (Schoon 1996, Furton and Myers 2001). Between every dog, the platforms were cleaned with vinegar (7%) - water mix (1:3) and training preceded after the platform was dry (Pompl et al. 1999, Arendash et al. 2001, Arendash et al. 2006). Ensuring that the platform was clean from residual odors, saliva and other possible disturbances and cues from the other dogs or handlers (Papet 2016). The door between the two rooms was opened for a minute or two, for airing in between the dogs.

During training and experiments (See below) a dog could respond to a sample in four different ways: 1) a true positive (TP) response, the dog does a TFR on the target odor, i.e. a correct response, 2) a false positive (FP) response, the dog does a TFR on a non-

target odor, i.e. an incorrect response, 3) a true negative (TN) response, the dog returns to handler in the absence of the target odor or correctly reject non-target (phase one and two), i.e. a correct response and 4) a false negative (FN) response, the dog is returns to handler in the presence of a target odor, i.e. an incorrect response (Fjellanger et al. 2002, Furton et al. 2010, Concha et al. 2014, Jezierski et al. 2015, Jezierski 2016).

2.3.1.1. *Phase one*

Phase one was split into two main goals: 1) adaption to the laboratory and table platform and 2) initial training. Only the grosspitz underwent goal one, as the dog was naïve to the environment, any kind of scent detection work and the table platform. The remaining four dogs had been taught the passive response, lay down, as the TFR (Furton et al. 2010, Hurt et al. 2016) and started directly on goal two. By using a passive response, the dogs are showing us a clear and non-mistakable indication and simultaneously they are less likely to destroy or contaminate a vulnerable sample if they later would become trained to work outside in the field (Furton et al. 2010, Hurt et al. 2016).

In phase one and two, a training session consisted of five trials, and each trial of ten randomly chosen lineups per dog using a random number generator (Excel), i.e. one session equals five trials and therefore fifty lineups. If a dog failed to do a TP response on the target sample, the handler did not move the samples until the dog did a TP response on the correct sample. Then the handler moved the samples to yet a random location, and the trial continued. In the respective phases, there was always one target sample among six control sample in every trial.

To teach the grosspitz the TFR the handler placed seven sterilized plastic cups in the table platform, with dog treats in the one plastic cup connected to the handle (target sample) (Fig 1A). The handler simply started with the command "search" and pointed in the direction of the samples, the dog started sniffing each hole, and when it discovered the dog treat, there was an instant change in behavior. In the beginning it was tolerated that the dog was pointing with its nose and standing still in the direction of the sample, but after a few correct responses and noticing that behavior the handler told the dog "down", resulting in a click and a following reward when expected behavior was made. Caution was made to ensure that the dog learned the context with laying down when discovering the treat odor, a click and delivery of a reward and not just performing a behavior to get

a reward. The TFR was expected to last one second to get a reward in the beginning of the training period, and later prolonged for at least three seconds (Fischer-Tenhagen et al. 2011). After the dog independently sniffed all samples in the platform and performed 100% correct in 3 consecutive sessions, the adaption was completed, and the dog (Akira) carried on to goal two.

Same method was used for the initial training on the table platform: one target sample (Rock ptarmigan) and six control samples in the table platform. The goal was to teach the dogs to recognize Rock ptarmigan and to discriminate it from control samples used during sample preparation and training. One trial could consist of one target sample, one plastic jar with silica gel, one empty plastic cup, one plastic cup with a disposable glove, one plastic cup with an empty glass vial, one plastic cup with an empty glass vial with identical marking as the fecal pellets and one blank. The initial training was completed when all dogs performed within the threshold values of at least 80% correct responses in three consecutive sessions (Fischer-Tenhagen et al. 2011).

2.3.1.2. Phase two

Phase two continued with the discrimination training that started so dilatory in phase one and was also divided into two goals: 1) discrimination training with Western capercaillie and Black grouse, and 2) discrimination training with Willow ptarmigan. Fecal pellets from Western capercaillie and Black grouse was added to the table platform as control samples. Now, one trial could consist of one target sample, one Western capercaillie, one Black grouse sample and four other randomly chosen controls. A trial within goal two could consist of one target sample, one Willow ptarmigan sample, one Western capercaillie sample, one Black grouse sample and three other randomly chosen controls. Phase two was completed when all dogs performed with at least 80% correct responses in five consecutive sessions, in both goals.

2.3.1.3. Phase three

Phase three was an adaption from the table platform to a one-holed platform by using a two-holed platform as an intermediate step and simultaneously teaching of a TN response, and was split into four goals: 1) teaching of a TN response, 2) training on a four-holed platform, 3) training on a two-holed platform and 4) training on a one-holed platform. One session consisted of six trials, one random lineup per trial. By adding zero trials to the training, trials with no target sample, the possibility of the dogs laying down in front

of the platform when no target sample was present decreases, thus they will experience higher reliability and a lesser amount of false responses (Schoon 2002). A zero trial could consist of a lineup with a blank or the Willow ptarmigan.

To teach the dogs a TN response the table platform with four available holes was used and the handler was placed two meters in front of the platform instead of standing behind it because of the "Hans effect" (Pfungst and Rahn 1911). One trial could consist of no samples, or with a Willow ptarmigan sample in one of the four holes. To increase the dogs motivation and make them feel more success, the trained no-decision was equally rewarded as the yes-decision (Gazit et al. 2005, Johnen et al. 2017). In the beginning of teaching them a TN response, the platform was empty. The training started with the command "search" and the dogs started sniffing each hole searching for a target sample, when no response was made, the handler called the dogs and they returned to handler resulting in a click and a reward. After five sessions mixing trials with no target samples and trials with target sample, they learned the context of laying down in front of the target sample and returning to handler when absent.

After teaching a TN response, goal two started with the table platform presenting the dogs with four holes. One trial could consist of no target sample, thus four control samples, or one target sample (Rock ptarmigan) and three control samples. From here on, all training was carried out in a single blind manner (Johnen et al. 2013, Kardish et al. 2015, Traniello and Bakker 2015). The experimenter was placed in the observer room watching the dog-handler team through a video monitor. Both the handler and the experimenter were equipped with a clicker, and the sound of a click could be heard between the two rooms with the door locked. The handler had to confirm the dogs respond by performing a hand gesture, if the team was correct, a click from the experimenter and handler was provided and the dog rewarded. If the team was incorrect, a click was not provided, and they exited the training room with a neutral behavior from the handler.

Training procedure with two-holed and one-holed platform, hence goal three and four, was equal to training with four-holed platform except the dogs were presented with two or one options at the time, all randomly chosen. By using the two-holed platform, the dog could be presented with four alternative lineups: 1) Rock ptarmigan and blank, 2) Rock ptarmigan and Willow ptarmigan, 3) Willow ptarmigan and a blank or 4) empty platform.

By using a single-holed platform, the dog could be presented with three alternative lineups: 1) Rock ptarmigan, 2) Willow ptarmigan or 3) blank. Phase three was a more dynamic phase than the previous phases, and there was need to go back steps and change the training regime in relation two- or four-alternative choices, to ensure greater motivation and better result for the detection-teams (Porritt et al. 2015). With time as a restriction, threshold values were adjusted to respective 70% correct responses in five consecutive sessions, to move on to the final experiments. Due to sickness, one dog (Tapas) was not able to complete phase three and the final experiments (See below).

2.4. Final experiments

Final experiments were undertaken within an eight-week period between 10th of January to 14th of March 2018, and six experimental days was carried out. Two females were experiencing heat in one session each, waiting outside from the laboratory until the other dogs finished the session, completing the experiment using panties with padding. Experimental procedures and set up were equal to the last goal in phase three, except all sessions were carried out in a double blinded matter (Kardish et al. 2015, Traniello and Bakker 2015, Johnen et al. 2017). Equal number of positive and negative samples were used to ensure that the possibility of a sample to be a target samples was 50% and by using a varying number of positive and negative samples in a session the handler and dogs could not follow certain patterns and give accidental cues (Jezierski 2016).

A third person, an observer not invested in the study, chose both samples and the order using a random number generator, and they were all used once for each dog (Elliker et al. 2014, Jezierski 2016, Johnen et al. 2017). When a sample was placed inside the platform, it was waited two minutes for the sample to set and the platform to dry off (Concha et al. 2014). Dog-handler team then entered the experimental room waiting for a start signal from the experimenter watching the video monitor in the second room. The experimenter was in direct contact over a telephone with the observer, placed in a car next to the laboratory. By using this kind of double-blind testing, we were able to directly reinforce the dog when it performed a correct response and no reward when incorrect (Jezierski 2016). All trials were videotaped using two tripods cameras placed in the two corners of the room, whereas one was pointing against the platform and the other one at the handler, the tapes were watched, and responses confirmed by the observer after the experiments

were done to ensure no observational bias (Kardish et al. 2015, Traniello and Bakker 2015).

2.5. Data analysis

Three parameters were calculated to evaluate all dogs from the four possible responses: sensitivity, specificity and accuracy (Concha et al. 2014, Jezierski et al. 2015, Oh et al. 2015):

Calculation of sensitivity: TP / (TP + FN)

Calculation of specificity: TN / (TN + FP)

Calculation of accuracy: (TP + TN) / (TP + FP + TN + FN)

Chi-square goodness-of-fit test was calculated using a contingency table to establish if 1) the dogs' responses were significantly ($p < 0.05$) higher than expected by chance, i.e. the probability that a random sample is a target sample is 0.5 and 2) is there a significant difference ($p < 0.05$) between number of responses made by the dogs on a sample and the correctness of that sample, i.e. there is a greater possibility of a sample to be correct if four dogs are doing a TFR on a sample, compared to if one dog does a TFR on a sample.

By using the description in Schoon et al. (2014), a system approach that are allowing the dogs to function as a system (rather than individuals) and their common responses providing a system-evaluation of a single sample at the time, was set up. The system outcome was based on their average false positive ((FP)/(FP + TN), analogous to type one error) and negative rate ((FN)/(FN + TP) analogous to type two error), their average true positive (sensitivity) and negative rate (specificity) and the result of the chi-square goodness-of-fit test (2) (Schoon et al. 2014, Gadbois and Reeve 2016). A sensitive system was emphasized, and ≥ three dogs were required to perform equal response (either lay down or return to handler) on an arbitrarily sample for it to be consider correct. A negative system outcome "*low suspect of a sample to be correct*", i.e. need further identification of species through DNA analysis, was considered if the sample had 1-2 equal responses on a sample (lay down or return to handler). Positive outcome "*High suspect of a correct sample*", i.e. most likely fecal pellets from the Rock ptarmigan if ≥ three or more dogs performed a response where they laid down, or control if ≥ three or more returned to handler.

3. Results

3.1. Training

All dogs who completed all training phases, finished with an average of 81 (SD ± 3.4) sessions (Tab. 1). Among all the dogs phase one was completed within an average of five sessions, phase two with an average of 24 sessions and phase three with the four finishing dogs with an average of 51 sessions. All dogs completed all goals and phases within the thresholds values with an average sensitivity, specificity and accuracy of 90%, 97% and 95% in phase one, 85%, 95% and 92%, respectively in phase two, and 58%, 81% and 75% in phase three (Tab. 2).

Table 2. Result presented as sensitivity, specificity and accuracy in percent from all dogs in all training phases and goals. Calculated from result achieved in the five last consecutive sessions in each goal in the respective phases, except phase one, goal one where the result was calculated from result achieved in the last three consecutive sessions.

Phase one			**Dogs**			
Goal one	**Akira**	**Bailey**	**Chilli**	**Shib**	**Tapas**	**Average (%)**
Sensitivity	100	-	-	-	-	**100**
Specificity	100	-	-	-	-	**100**
Accuracy	100	-	-	-	-	**100**
Goal two						
Sensitivity	88	89	81	75	69	**80**
Specificity	96	96	94	92	90	**94**
Accuracy	94	94	91	88	84	**90**
Phase two						
Goal one						
Sensitivity	90	87	82	91	82	**86**
Specificity	97	96	94	97	94	**96**
Accuracy	95	93	91	98	91	**93**
Goal two						
Sensitivity	80	80	83	86	86	**83**
Specificity	93	93	94	95	95	**94**
Accuracy	90	90	91	96	90	**91**
Phase three						
All goals						
Sensitivity	57	41	62	71	-	**58**
Specificity	72	87	76	90	-	**81**
Accuracy	71	73	75	81	-	**75**

3.2. Final experiments

Each dog carried out 36 double-blind and randomized trials (Appendix 2), and they recognized and discriminated Rock ptarmigan from the controls with an average accuracy of 66% (SD ± 10), sensitivity of 67% (SD ± 26.1) and specificity of 65% (SD ± 18.5). The result revealed a considerable range between the poorest and strongest performing dog in sensitivity (33% and 94%), specificity (44% and 89%) and accuracy (61% and 81%) respectively (Tab. 3). All dogs performed better than expected by chance with 95 correct responses of a total of 144 possible (X^2 =12.6, df =1, p < 0.001) (Tab. 3) and there was a significant difference between the number of responses on a sample and the correctness of a sample (X^2 = 14.73, df = 4, p < 0.05).

Table 3. Result presented as sensitivity, specificity and accuracy in percent and number of responses from the four evaluated dogs calculated from 36 trials divided in 6 experimental sessions. TP = true positive response, FP = false positive response, TN = true negative response, FN = false negative response, CR = correct responses, IR = incorrect responses.

Dog	Trials	TP	FP	TN	FN	CR	IR	Sensitivity	Specificity	Accuracy
Akira	36	14	10	8	4	22	14	78	44	61
Bailey	36	6	2	16	12	22	14	33	89	61
Chilli	36	13	6	9	8	22	14	62	60	61
Shib	36	17	6	12	1	29	7	94	67	81
Total	**144**	**50**	**24**	**45**	**25**	**95**	**49**	-	-	-

3.2.1. System evaluation

13 times did ≥ three dogs lay down in front of the platform, and 10 of those was the Rock ptarmigan present (76%), but in total the dogs only laid down in front of the platform 10 out of 18 times when the Rock ptarmigan was present (55.6%) (Fig. 2).

12 times did ≥ three dogs return to handler, and 10 of those times was the Rock ptarmigan absent (83%), but in total dogs only returned to handler 10 out of 18 times when the Rock ptarmigan was absent (56.6%) (Fig. 2).

The system was able to identify 20 out of 36 samples in total with a certainty of 80%, (≥ three dogs laid down or returned to handler 25 times, and 20 of them was correct) which reduced the cost with species identification through DNA-analysis with 55.6% (Fig. 2).

Akira	Bailey	Chilli	Shib	Fecal pellets Rock ptarmigan	≥ three equal responses (lay down)	Fecal pellets Willow Ptarmigan or blank	≥ three equal responses (return to handler)	Correct evaluations
R	R	D	R					
D	R	R	D					
R	R	R	R					
R	D	D	D					
D	R	R	D					
R	R	R	R					
D	R	D	D					
D	D	D	D					
D	D	D	D					
D	D	D	D					
D	R	D	D					
D	D	D	D					
R	R	R	D					
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D	R	R	R					
D	D	D	D					
D	R	D	D					
D	D	D	D					
D	R	R	D					
R	R	R	D					
R	R	D	R					
D	D	R	R					
R	R	D	D					
D	D	D	R					
D	R	D	D					
R	R	D	R					

Figure 2. System evaluation of result. The column "Fecal pellets Rock ptarmigan" is the actual number of Rock ptarmigan samples in the final experiment (18/36 samples). The column called "≥three equal responses (lay down)" is number of times three or more dogs laid down in front of the platform. Column "Fecal pellets Willow ptarmigan or blank", is the actual number of Willow ptarmigan samples and blanks in the final experiments (18/36). The column "≥ three equal responses (return to handler)" is number of times three or more dogs returned to handler. Column "Correct evaluations" is the number of times the dogs as a system was correct.

4. Discussion

The hypothesis in this study was supported, dogs can be trained to recognize fecal pellets from Rock ptarmigan and discriminate it from the Willow ptarmigan. Training results suggests that our dogs were able to learn the odor from the Rock ptarmigan quite fast, and successfully discriminated it from the Willow ptarmigan within phase two, with an average of 29 sessions using the table platform with high sensitivity and specificity (Tab. 2). All dogs performed significantly better than expected by chance ($p < 0.001$) in the final experiments and a statistical test shows that there was a significant ($p < 0.05$) difference between number of dogs performing a TFR (Laying down or returning to handler) on a sample, and the correctness of that sample. Result through the system approach reveals that cost on species identification through DNA-analysis may be reduced with up to 55.6%. The system approach seems to be advantageous tool in future laboratory conditions

This study reveals that there must be a difference in the chemical composition of fecal pellets from the close related avian species. Both species inhabit slightly similar habitats, they may experience habitat overlap (Watson and Moss 2008, Quintela et al. 2010), and they consume slightly similar food with an exception of the winter diets if both the Rock ptarmigan and the Willow ptarmigan occur sympatric (Watson and Moss 2008). The most suitable explanation would be the difference in their genetics (Gyllensten et al. 1985, Sveinsdóttir and Magnússon 2017).

Ideally, detection dogs should both detect and discriminate the target from other non-targets with an accuracy of 100%. However, in reality there are several factors that can influence the dog-handler teams detection and accuracy rate (Gadbois and Reeve 2016, Johnen et al. 2017). A working dog have been stated to have increased success when it comes to carefully selection in relation to breed and personality traits as e.g. high trainability, high drive (hunt-, prey-, play- and motivational drive), useful amount of independence, and fitness (Rebmann et al. 2000, Furton et al. 2010, Beebe et al. 2016, Jamieson et al. 2017). None of our dogs were selected in case of these personality traits, but rather as convenience. An important factor to document, as many of today's studies are based on highly successive working dog with long experience and a random dog may not be capable of achieving their detection skills (Fjellanger et al. 2002, Smith et al. 2003, Wasser et al. 2004, Gadbois and Reeve 2016). Studies have shown that pet dogs

demonstrated higher dependencies towards their owners when it came to novel situations or problem solving compared to exclusive working dogs (dogs who did not live with their caregivers) (Topál et al. 1997). Topál et al. (1997) stated that the reason for that is their social depended behavior causing strong bonds to its family and occasionally looking for reassurance and encouragements. However, studies using privately owned pet dogs not selected for their desired detection traits, have also shown successful detection rate with high accuracy (Fischer-Tenhagen et al. 2011, Johnen et al. 2013, Fischer-Tenhagen et al. 2017, Rosell et al. Submitted). A dog fulfilled with the respective and desired properties is dependent on a suitable handler to be a fully successive dog-handler team (Smith et al. 2003, Furtado et al. 2008, Beebe et al. 2016). A suitable handler can both understand and confirm his/hers dogs behavior and with knowledge in dog training (Gutzwiller 1990, Smith et al. 2009) and the success or failure of the dog-handler team is largely dependent experience and chemistry between them (Gutzwiller 1990, Smith et al. 2003, Cablk and Heaton 2006). No handler in this study was professional handler nor dog trainers, but always had a genuine interest in dogs and dog training.

This study reveals a higher false positive and false negative rate than ideal. Using dogs not selected for their desirable working traits and unexperienced handlers, several issues may encounter (Smith et al. 2003, Hurt et al. 2016). There was followed reward methods described in Gadbois and Reeve (2016), Fischer-Tenhagen et al. (2017) and Johnen et al. (2017) and both TP- and TN-responses was rewarded. Using a yes/no training regime, there is a 50% chance of an arbitrarily sample to be correct and just by guessing the dogs would have a considerable chance of getting a reward. Fischer-Tenhagen et al. (2011) stated that dogs that are used with clicker-based training, may in novel situations perform different kinds of behaviors to get a reward. Hurt et al. (2016) addressed issues as e.g. "lying", often seen with dogs lacking ideal work ethic or training endurance, which result in a higher false alert rate, where the dogs are skipping the hard part of the training to get to the rewards. Target confusion may also be a possible issue for high false positive and false negative rates, generally caused by insufficient amount of training, where the dogs do not know how to generalize all variants of the target (Hurt et al. 2016). The sample collection in this study consisted of fecal pellets that had been dried and stored in a fridge with silica gel for up to one and a half years before they were used in dog training. There was a considerable chance that the fecal pellets had lost a considerable amount of their original content of volatile compounds and DNA quality. Wasser et al. (2004) and Smith

et al (2003) found that the older scats had significantly lower DNA quality than fresh scats (zero days to a month or less than eight days).

Result in training phase three and the final experiments indicates that training on a one-alternative choice platform with a yes/no training regime tended to be more challenging for our dogs than training on a multi-alternative platform (table platform), also stated in Gadbois and Reeve (2016). A one-alternative choice set up would increase the difficulty of the detection task, simultaneously as it would give a good overview of the dogs biases and are allowing the handler to decrease them (Gadbois and Reeve 2016). A multi-alternative choice set up with more than three alternatives, i.e. scent lineups where dogs are choosing a target sample among several non-target samples (Fjellanger et al. 2002, Fischer-Tenhagen et al. 2011, Gadbois and Reeve 2016), have been stated to increase the sensory and mnemonic interference in dogs (Gadbois and Reeve 2014, 2016). However it will also reduce performance of the dogs as the probability of the next sample to be a target will change throughout the lineup if it is not the first sample to encounter (Gadbois and Reeve 2016, Fischer-Tenhagen et al. 2017). In addition there is speculation of dogs using non-targets to compare samples to find the target sample (Gadbois and Reeve 2016, Fischer-Tenhagen et al. 2017). In platform training, there will always be a possibility that dogs are learning the specific target sample already after a few encounters, causing dogs not to learn the generalized odor perception of the target, but rather individual scents present in the lineup (Fischer-Tenhagen et al. 2011). In a yes/no training regime, dogs will only encounter a sample once, with no other sample comparison, making it a pure detection task (Gadbois and Reeve 2016, Fischer-Tenhagen et al. 2017). However, considering our result and comparing it with other previous studies with similar training regime, the average detection accuracy, sensitivity and specificity rate of 73.8%, 56.5% and 91.5% (Gadbois and Reeve 2016) and 75%, 72% and 84% respectively (Fischer-Tenhagen et al. 2017) are in accordance with the result in this study, not indicating large differences.

Looking at the individual dogs, Shib showed a high detection accuracy and ability to discriminate between the two species, the dog showed a high motivational factor and was thorough in her decision making throughout the entire process. The three other dogs showed periodically lack of motivation, often seen in highly repetitive and unilateral tasks (Porritt et al. 2015). Akira was more accurate at detecting the Rock ptarmigan, but less

accurate at indicating when the Willow ptarmigan was present. Bailey was less accurate at detecting the Rock ptarmigan, but more accurate at indicating the presence of Willow ptarmigan, i.e. indicating that Rock ptarmigan was not present. While Chilli showed a more similar detection accuracy of both detecting the Rock ptarmigan and indicating the presence of the Willow ptarmigan.

As far as I know, no study has investigated the potential use of dogs to discriminate between avian species in the same genus, and few studies have investigated the potential of dogs to discriminate between mammals within the same genus. Rosell et al. (Submitted) trained dogs in laboratory conditions to discriminate between the two beaver species, the Eurasian beaver and the North American beaver. They trained dogs using 15 years old castoreum samples collected from dead beavers (sensitivity = 90% and specificity = 98%). As a second olfactory test, they investigated the potential of dogs to recognize and discriminate scent marks collected from living beavers with high detection accuracy (Sensitivity = 85% and specificity = 94%) and successfully demonstrated that samples can be brought from the field and into laboratory conditions for further research. This study also reveals that dogs are able to learn more heavily volatile compounds in a biological sample, as the dogs correctly identified fresh castoreum samples collected from the wild, when only have been trained on castoreum samples collected from beavers that had been stored in a freezer for 15 years and probably have been experiencing loss of the lighter volatile compounds. Smith et al. (2003) trained dogs in controlled conditions to discriminate San Joaquin kit foxes from other sympatric fox species. They brought the dogs into the field and they successfully discriminated kit fox scat from other fox scats (100%) in both controlled discriminations conditions and field search but showed reduced accuracy (67%) rate when the kit fox scat was absent, i.e. it was more difficult for the dogs to ignore the sympatric fox species when the kit fox scat was not present. All dogs showed an ability to ignore other sympatric species that was not tested for during training as well, as e.g. coyotes, striped skunks (*Mephitis mephitis*) and American badgers (*Taxidea taxus*). It is difficult to directly compare result with the two respective studies because of the differences in training regime, where Rosell et al. (Submitted) used both a multi-alternative platform with six hole, and a platform with three holes. While Smith et al. (2003) used a "scent line" consisting of a variety of five to ten scent boxes and in addition the field based experiments.

There is no standardized methods for surveying the Rock ptarmigan, point-transect with registering of calling activity from adult males are most commonly used estimating through analyzing of occupancy and distance models, but are often providing a non-realistic and non-certain estimation of a population based on the assumption of a ORS relationship of 1:1 (Carvalho et al. 1998, Marty and Mossoll-Torres 2012). However, collecting of fecal pellets will provide the researcher with a lot of information about the species/population investigated, as e.g. sex (Putman 1984, Kohn and Wayne 1997, Wasser et al. 2004). Ways of collecting these samples have proven to be is time-consuming, costly and slightly inefficient, simultaneously requiring an additional amount of time in the laboratory after collecting the samples to complete the analysis. Studies have shown dog-human teams to decrease bias, be more accurate and efficient to collect fecal matter in relation to time and area (size) searched compared to human-only teams (Wasser et al. 2004, Arandjelovic et al. 2015, Cristescu et al. 2015), and compared to hair snares and cameras (Long et al. 2007). Even though our dogs are trained in laboratory conditions, with some further field search training, there is no reason to believe that they cannot be surveying in field as a non-invasive tool to find fecal pellets from the Rock ptarmigan as previous study have reported that dogs were able to find target in the field after laboratory conditions (Smith et al. 2003, Gadbois and Reeve 2014). However, dogs have shown difficulties being brought back to laboratory conditions after high stimulated field search, because of lack of motivation (Gadbois and Reeve 2014).

As climate is gradually changing (Thomas 2010) and human disturbances increases (Bevanger and Brøseth 2000, Viñuela and Arroyo 2002, Watson and Moss 2004), the Rock ptarmigan and the Willow ptarmigan is expected to share a greater amount of habitat (Viñuela and Arroyo 2002, Storch 2007, Watson and Moss 2008, Quintela et al. 2010). The most accurate method to distinguish the Rock ptarmigan from the Willow ptarmigan, is identification of species through DNA-analysis, method considered to be both time-consuming and expensive. Our system approach show that dogs can be used to evaluate fecal pellets, and that it can reduce both cost and time as the result is immediate. Bringing field samples in to laboratory conditions is a well known phenomenon in mine detection dogs and situations where dogs and handler may be in danger entering field (Fjellanger et al. 2002, Gadbois and Reeve 2014). The method has proven to give many advantageous, as e.g. controlled microclimate, optimized scent perception by immediate delivery of rewards and controlled and familiar environment (Gadbois and Reeve 2014).

However, training detection dogs is also time-consuming and expensive, but when they are proficiently trained, little maintenance is required, once a week have proven to be enough (Horvath et al. 2013), and the result in the laboratory is immediate. Partnership with public agencies or with e.g. police will reduce the cost of a trained detection dog dramatically as the dog already is trained with scent in focus (Orkin et al. 2016).

4.1. Conclusion

Ideally more dogs should have contributed to the study, minimizing the individual dogs' performance (Fischer-Tenhagen et al. 2011, Johnen et al. 2017, Fischer-Tenhagen et al. 2018). Several factors can influence the accuracy of a detection dog, but with thorough understanding of possible biases and carefully considerations of dogs and handlers, a dog-handler team with an acceptable success rate may be achieved. The dogs in this study demonstrated that they can detect differences between the close related avian species via fecal pellets and the result suggests that dogs can be used as biodetectors, a tool to recognize the Rock ptarmigan and discriminate it from other bird species in the *Tetraonidae* family (Black grouse, Western capercaillie and Willow ptarmigan). The system approach can serve as an additional tool to fecal analysis providing management and researchers with information about the species, cooperating with manufacturing of conservation plans and measures to help endangered species. Costs and time in both training and maintaining proficiency in detection dogs may be reduced by establishing collaboration with public agencies.

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Appendix

Appendix 1: Site nr, site name, county and number of samples from that particular site on the fecal pellets

Site nr	Site name	County	Samples (n)
Site 1	Sørbøfjellet, Flå	Buskerud	35
Site 2	Blefjell	Buskerud / Telemark	28
Site 3	Norefjell / Reinsjøfjell	Buskerud	46
Site 7	Nystølfjellet, Gol	Buskerud	9
Site 8	Synnfjell, Nordre Land	Oppland	28
Site 9	Langsua, Gausdal	Oppland	22
Site 12	Femundsmarka	Hedmark / Sør-Trøndelag	29
Site 14	Tronfjellet, Alvdal	Hedmark	39
Site 19	Dalegubben, Ørsta	Møre- og Romsdal	7
Site 25	Skenörsfjellet	Jämtland (Sweden)	27
Site 26	Kjølifjellet, Holtålen	Sør-Trøndelag	6
Site 28	Oviksfjällen	Jämtland (Sweden)	49
Site 29	Roan, Fyresdal	Telemark	14
Site 30	Bykleheiane, Bykle	Aust-Agder	35
Site 32	Hallingsskarvet	Buskerud	19
Site 33	Aursjø, Dovrefjell	Oppland / Møre- og Romsdal	5
Site 34A	Andbergshøe, Dovrefjell	Oppland	13
Site 34B	Reinheimen, Lordalen	Møre- og Romsdal	20
Site 37	Stavsfjell, Vinje	Telemark	16
Site 39	Slagsfjella, Gausdal	Oppland	17
Site 40	Ringebufjella, Ringebu	Oppland	4
Site 41	Storhøgda, Rendalen	Hedmark	14
Site 42	Nipfjället	Dalarna	15
Site 43	Fulufjellet, Trysil	Hedmark / Dalarne (Sweden)	25
Site 44	Vedungfjällen	Dalarna	24
Site 45	Sånfjället	Jämtland (Sweden)	25
Site 46	Skorvdalsfjället, Björnrike	Jämtland (Sweden)	23
Site 48	Sylan	Sør-Trøndelag / Jämtland (Sweden)	41
Site 50	Ilfjellet, Rennebu	Sør-Trøndelag	19
Site 52	Vikbekkfjellet, Tinn	Oppland	23

Appendix 2: Information about fecal pellets used in the final experiment

Experiment	Sample (nr)	Species	ID	Site	County	Sex	Year
1	13	L.l	37-61	Stavsfjell, Vinje	Telemark	X	2016
	127	L.l	07-25	Nystølfjellet, Gol	Buskerud	X	2015
	157	L.l	40-36	Ringebufjella, Ringebu	Oppland	X	2015
	50	L.m	48-02	Sylan	Sør-Trøndelag / Jämtland (Sweden)	M	2016
	3	L.m	45-07	Sånfjället	Jämtland (Sweden)	M	2016
	-	Blank	-	-	-	-	-
2	135	L.m	01-40	Sørbøfjellet, Flå	Buskerud	M	2015
	116	L.m	50-10	Ilfjellet, Rennebu	Sør-Trøndelag	F	2015
	138	L.m	41-21	Storhøgda, Rendalen	Hedmark	M	2015
	108	L.m	14-20	Tronfjellet, Alvdal	Hedmark	M	2015
	153	L.l	08-01	Synnfjell, Nordre Land	Oppland	X	2015
	-	Blank	-	-	-	-	-
3	4	L.m	45-16	Sånfjället	Jämtland (Sweden)	M	2016
	129	L.l	07-07	Nystølfjellet, Gol	Buskerud	X	2015
	63	L.m	28-30	Oviksfjällen	Jämtland (Sweden)	M	2016
	142	L.l	37-01	Stavsfjell, Vinje	Telemark	X	2015
	113	L.l	14-36	Tronfjellet, Alvdal	Hedmark	X	2015
	-	Blank	-	-	-	-	-
4	74	L.m	50-02	Ilfjellet, Rennebu	Sør-Trøndelag	M	2015
	155	L.l	08-39	Synnfjell, Nordre Land	Oppland	X	2015
	140	L.m	37-04	Stavsfjell, Vinje	Telemark	F	2015
	92	L.m	43-02	Fulufjellet, Trysil	Hedmark / Dalarne (Sweden)	M	2015
	72	L.l	48-46	Sylan	Sør-Trøndelag / Jämtland (Sweden)	X	2016
	-	Blank	-	-	-	-	-
5	69	L.l	48-43	Sylan	Sør-Trøndelag / Jämtland (Sweden)	X	2016
	58	L.m	28-17	Oviksfjällen	Jämtland (Sweden)	M	2016
	36	L.m	52-17	Vikbekkfjellet, Tinn	Oppland	M	2016
	59	L.m	28-08	Oviksfjällen	Jämtland (Sweden)	M	2016
	65	L.m	28-43	Oviksfjällen	Jämtland (Sweden)	M	2016
	-	Blank	-	-	-	-	-
6	164	L.l	40-08	Ringebufjella, Ringebu	Oppland	X	2015
	76	L.m	25-34	Skenörsfjellet	Jämtland (Sweden)	M	2015
	39	L.m	52-07	Vikbekkfjellet, Tinn	Oppland	M	2016
	23	L.l	02-10	Blefjell	Buskerud / Telemark	X	2016
	88	L.m	39-14	Slagsfjella, Gausdal	Oppland	F	2015
	-	Blank	-	-	-	-	-

X = unknown sex
Year = year collected in field
L.l = Lagopus lagopus
L.m = Lagopus muta