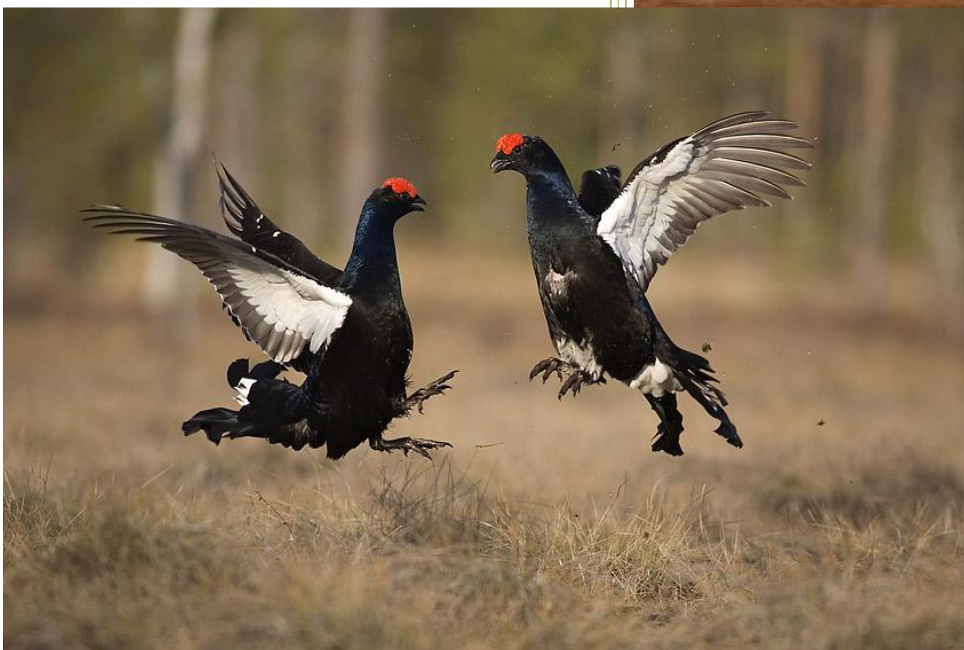
2016

Reintroduction and GPS tracking of the black grouse in the National Park De Hoge Veluwe



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Summary

In 2007, the National Park De Hoge Veluwe started reintroducing the black grouse (*Tetrao tetrix*) into their park. Until now, the reintroduction was not very successful. The survival rates are low and there is no prove of reproducing black grouses in the park. To monitor the movements and habitat use of the released grouses, some of the grouses will receive a GPS transmitter before they are released. However, the effects of transmitters on ground dwelling bird species, including the black grouse, are not clear yet. This study investigated the effects of GPS senders attached by a harness on captive male and female black grouses. To investigate whether the transmitter has an influence on the behavior of the black grouses, the grouses were observed before and after the attachment of the transmitter. Some changes in behavior occurred after the birds received their transmitter, for example the increase in pacing, the decrease in courtship behavior and the birds were not able to fly the first few days. Also the effect on health and feather condition was examined and it seemed that the harness and sender did not have any negative influence on the health, weight or feather condition of the grouses. However, the sample size in this study was very small and the observations took place in different times of the year. Therefore, further research with a cross-over design and a bigger sample size is necessary to determine the true effect of a transmitter on black grouses.

Introduction

General background

The black grouse (*Tetrao tetrix*) is a large game bird belonging to the grouse family (*Tetraoninae*) in the order of Galliformes. Grouses are characterized by their feathered legs. In the winter, their toes can have feathers or small scales on the sides, allowing them to walk in the snow. Black grouses are sexually dimorphic; blackcocks are considerably larger and more colorful than greyhens (Smit, 2003). Black grouses are habitat specialists and have a complex social system. They mainly live in and around young woods, especially in heath- and peatlands. The birds depend on temporary stages of the vegetation succession. They feed on seeds, leafs, berries, pollen cones, fresh sprigs, grasses, herbs and buds of plants, trees and conifers. Chicks of the black grouse, mainly, eat invertebrates, caterpillars and larvae (Jansman *et al*, 2014; van der Ziel & van der Lans *et al*, 2004).

The black grouse used to exist in all of Europe, however, nowadays it is red-listed in most European countries (Segelbacher *et al*, 2014). In 1950, black grouses were found in the north-western part of Europe, from Belgium, the Netherlands and Germany in the west to Denmark in the north and Poland in the east (Niethammer, 1966). The number of black grouses in the Netherlands decreased with 90% in the last 60 years (Larsson *et al*, 2008; Ludwig *et al*, 2008). Before 1940, 5000-8000 cocks lived in the Netherlands and around 1990 only 30 cocks were left (Larsson *et al*, 2008). Today, only one small population still exists, in the Sallandse Heuvelrug.

The change of habitat is the main cause of the decline in black grouses. In the Netherlands, the biggest change was caused by intensification of agriculture. Reclamation led to the disappearance of the original peat- and heathlands. Young trees on heathlands turned into mature pines and deciduous forests. This caused a reduced suitability of habitat, since black grouses use heath- and peatlands with young trees as resting, nesting and foraging area (Angelstam *et al*., 2000; Baines *et al*, 2000; Swenson *et al*, 1993; Niewold 1993). Moreover, black grouses are vulnerable birds and sensitive to disturbance (Weyland, 1978; Zeitler, 2000). Repeated disturbance causes the habitat to shrink and recreation is thought to disrupt the breeding process, as black grouse are extremely shy of humans (Weyland, 1978).

Also predation pressure is an important factor in the decline of populations. Goshawks (*Accipiter gentilis*) are the main predators, red foxes (*Vulpes vulpes*) are the secondary hunters. Goshawks and red foxes were both hunted in the 20th century and goshawks were even nearly extinct in 1960. However, given that the grouse population nevertheless declined, it is unlikely that the extinction of black grouses is caused by predation alone (Smit, 2003).

The black grouse symbolizes an ecosystem of agrarian nature and is considered an indicator of a rich and varied heathland (Smit & Bos, 2008; Leidekker, 2014). The biodiversity of the agrarian nature ecosystem has declined in Europe, the last decennia (Storch, 2000). Therefore, the foundation Nationale Park De Hoge Veluwe (NPDHV) started reintroducing captive-bred black grouses in their park in 2007. It was first determined, whether the park would provide an adequate habitat for the black grouse (Smit, 2003), then a working plan was set up (van der Ziel & van der Lans *et al*, 2004). It was determined that 700 hectares of the Hoge Veluwe could form a suitable habitat and would provide enough food for the black grouse (Smit, 2003; van der Ziel & van der Lans, 2004). The habitat contains many well-developed and relatively old heather vegetation that could serve as a breeding habitat for the greyhens. The short grassy vegetation could be used as a lek for the blackcocks. There are plenty of nutritious and herbs meadows suitable as foraging areas (van der Ziel & van der Lans, 2004). Moreover, birds were able to survive winters, which provides evidence that the habitat of the NPDHV is suitable enough for black grouses (Leidekker, 2014).

The reintroduction of the black grouse has to be monitored conform the International Union for the Conservation of Nature (IUCN) guidelines (Smit & Bos, 2008). Monitoring was done in three different ways. Every spring, calling cocks were counted and from all spotted black grouses the date and location were noted. Additionally, until 2012 monitoring was done using radio transmitters (Leidekker, 2014).

Since 2007, at least 30 black grouses, bred in captivity, were released every year. In 2007, the first birds were released with the 'hard-release' method. Small groups of black grouses were released in different locations of the park. 38% of the birds were found dead, most likely slain by goshawks. Still, in April 2008 black grouses were spotted in the NPDHV, so some birds did survive (Smit & Bos, 2008). From the birds released in 2008, only a small number was found dead, soon after the release, and 2-5% still lived in the springtime of 2009. An improvement in survival could be because the release took place over a longer period of time and the different locations were further away from each other (Smit & Koopmans, 2009). A large amount of birds was released in 2010, a total of 180 black grouses. But mortality rates of the released birds are very high, 95-100% in the first two winters (Baines, 2013). In 2012, two 'soft-release' cages were built, located in the habitat of release. With the 'soft-release' method, the birds are housed in their future habitat for several weeks prior to release (see figure 1). This way, they can already get used to the environment. Also, hens and their chicks are released together (Leidekker, 2014). In 2011 the introduction program was evaluated. According to the spring counting's, there was no evidence for reproducing black grouses. However, black grouses were spotted in and out of the park multiple times through the year (Sierdsema *et al*, 2011).

Figure 1. The inside of the small part of a soft release cage.

While a healthy population can deal with predation, the high mortality rates of the released birds are likely caused by the main predators of the black grouse; the Goshawk and the red fox. Six breeding Goshawks pairs are located in the parks and approximately 15-20 fox dens are inhabited. There are, however, several other potential predators in NPDHV, including wild pigs (*Sus scrofa*), martens (*Martes*), stoats (*Mustela erminea*), badgers (*Melinae*), common buzzards (*Buteo buteo*), crows (*Corvidae*), common kestrels (*Falco tinnunculus*) and sparrowhawks (*Accipiter nisus*). These predators can hunt for nests of the black grouse. Hence, there are quite a lot of predators present in the park. As the numbers of reintroduced black grouses are relatively low, the loss of any individuals is of great impact to the success of the program. Therefore, it was advised in the working plan to control predation. For example, predators can be captured and released elsewhere, far from the habitat of the black grouse. They can also be lured away by feeding them at other sites or by releasing other prey for the predators to hunt (van der Ziel & van der Lans, 2004).

As mentioned before, the grouses were tracked before with a radio transmitter. However, with a radio transmitter, the grouses had to be followed in order to collect data. This was quite labour-intensive and it disturbed the released black grouses. Given the low resightings, the question was whether the birds might also leave the area of the NPDHV. Therefore, detailed information on the movement of the grouse is necessary. As radiotracking has several disadvantages, the management chose to deploy grouse with GPS loggers.

GPS tracking of black grouse

During this project, several birds received a GPS (Global Positioning System)-sender, to gain detailed information on habitat use of the black grouse. With the GPS data, the movements and habitat use of the birds can be monitored, which is an important requirement according to the IUCN reintroduction guidelines (IUCN, 2013).

Choice of equipment

To minimize negative effects of attached loggers on the animal, several aspects should be considered, such as weight of the sender, duration of the battery (to avoid re-capture) and method of attachment.

In the literature, the commonly accepted standard weight of an instrument should be less than 3% (or 5%) of the body mass of the involved animal. However, the basis for this rule is not entirely justified by scientific evaluation. The 5% rule originated from a suggestion by Brander et al. (1969) and was seen as standard and acceptable (Casper, 2009) for several years, even though there was no aerodynamic justification (Caccamise et al., 1985). But after a review of albatross and petrel studies, correlating the weight of transmitters with foraging trip durations and nest desertions (Phillips et al., 2003), the 3% rule arose. Nevertheless, it seems rather simple to create a rule based on just one review, especially since another review, that studied the negative effects of transmitters on birds, stated that the negative effects did not become more negative with heavier transmitters (Barron et al., 2010). Yet another study, which investigated the energetic consequences for birds carrying transmitters, stated that after the attachment of a device weighing 3% of the body mass, the mechanical power required for birds to fly at their minimum speed, increased with 5%. With a device weighing 5% of the bird's body mass, the mechanical power increased with 10% (Vandenabeele et al. 2012), therefore heavier instruments seem to have an exponentially stronger effect on bird flight. It is worth noting, that it might be inappropriate to apply one rule to all birds for multiple reasons: the amount of time different bird species spend flying differs (Vandenabeele et al. 2012), species have different fly modes (continuously flapping birds have higher energy expenditure than (partially) gliding birds) (Birt-Friesen et al. 1989; Klaassen 1996), and heavier birds are more affected by device mass because they have less 'power surplus' (Caccamise et al., 1985). Using a transmitter weighing 3% of the body weight of the bird seems legitimate, but further research is needed to determine an ideally species-specific, safe maximum weight.

The influence of transmitters on the behavior and condition of black grouses is not clear, yet. However, there are articles describing the effect of transmitters on birds in general. Barron et al. (2010) concluded that birds are significantly negatively affected by devices. The research investigated multiple aspects, like nest success, offspring quality, flying ability, foraging behavior, energy expenditure and survival rate. All aspects were negatively influenced, except for flying ability. Most effects were small, but birds with transmitters had large increased energetic expenditure and were much less likely to nest (Barron et al., 2010). On the other hand, it was stated that a heavy backpack of 90 gram – and therefore 5-6% of the birds weight – did not influence the health of capercaillies (*Tetrao urogallus*) negatively; "When shot in early autumn, the hens were in 'normal' physical condition, with minimal feather and skin abrasion from the harness, and they flew well." (Wegge, 2007). In another study, transmitters were attached on Red Knots (*Calidris canutus*) with a harnass and the mentioned behavior was monitored, but the reported results did not give further details on the changed behavior (Chan, 2015). Thus, some studies exist on the potentially negative influence of transmitters on birds, but none really investigated the behavior after attaching a transmitter. Moreover, specifically the influence of transmitters on black grouses is not yet investigated.

Given the need for more information on the impacts of transmitters and method of attachment on behavior of ground dwelling bird species, the black grouse specifically, this study investigated the effects of GPS senders attached by a harness on captive male and female black grouse. Senders were chosen based on recommendations in the literature, and birds were observed prior and post attachment. As mass and methods of attachment of transmitters are also determined by the battery life and method of charging, these aspects were carefully considered, as well. See methods for further details.

Ethical considerations

Re-introduction

Regarding animal welfare and ethics, it can be questioned, whether black grouses benefit from being bred and reintroduced in the NPDHV. A video discussing this issue (Slats, 2013), caused a lot of debate in the Dutch society. Before the release, the birds live in captivity. Moreover, some birds will receive a GPS sender before they are released into the wild. Applying the transmitter will be a stressful experience, since the birds are not domesticated. According to the mortality rates, black grouses do not live very long in the NPDHV. Chances are high that they will be killed by predators. Therefore, it might be that living freely on the NPDHV is a short and frightening experience for the black grouses. The question is whether the animals' discomfort outweighs the chance of establishing a new population of black grouse on the NPDHV. Therefore, animal welfare aspects of the current reintroduction programs will be discussed.

GPS transmitter

As the harnass might pose mild to moderate discomfort to the birds, an ethical application was sent for approval to the Dutch animal experimentation commission 'Dierexperimentencommissie' (DEC) and the Centrale Comissie Dierproeven (CCD). The DEC considered the application and the project received their favorable opinion. The application was then sent to the CCD for final approval. Upon approval, the CCD issued the project license, which is required by the Experiments on Animals Act law (Wod) to carry out tests involving animals.

To estimate the discomfort caused by the attachment of the senders, a pilot study was performed with birds in captivity. Through behavioral observations we examined, whether the senders cause discomfort for the black grouses. This pilot study is also applicable to other reintroduction projects. If it turns out that the GPS transmitters cause moderate to severe discomfort, the ethical approval should be carefully considered in relation to the scientific value of the project. If the discomfort can be kept at mild, which involves the capture and handling of the birds, as well, transmitters can and should be used to gain knowledge about free-ranging birds and their habitat use. Nevertheless, further refinement of the methods can take place in any case.

Research hypothesis

Regarding behavior, it is to be expected that black grouses will experience mild discomfort after they receive the GPS-tracker, but habituation will occur and the difference in behavior will flatten out. After habituation, which we expected to take ca. 1-2 weeks, the birds should not experience discomfort anymore.

Regarding the physiological measurements, some effects might only become apparent over a longer time course, exceeding this study, e.g., feather damage, nevertheless, immediate damage can also occur. General health and bodymass are commonly used to assess animal welfare and can respond quickly to stressful circumstances. Therefore, all of these aspects were considered in this study. The indication of (long-term) negative effects in any of these aspects would have led to rejection of the attachment method.

Regarding the short-term effects on behavior and the possible long-term effects on physical health, the research hypotheses are split in two:

H0: The attachment of a transmitter via a harness does not influence the behavior of black grouses during the first week after attachment.
H1: The attachment of a transmitter via a harness does influence the behavior of black grouses during the first week after attachment.

H0: The attachment of a transmitter via a harness does not influence the health, feather condition and weight of black grouses three weeks after attachment.
H1: The attachment of a transmitter via a harness does influence the behavior, health, feather condition and weight of black grouses three weeks after attachment.

Material and method

Animals

For the pilot study two male and two female yearling black grouses were used. The birds were bred at the facilities of the National Park De Hoge Veluwe. During the observations, the black grouses were kept in the small part of a soft release cage. The soft release cage is located on heath land and is provided with several pine trees. The birds were fed grain and pellets for pheasants, and branches of larchs (Larix). The birds had ad libitum access to water, provided in a drinking bowl. Both feed and water were refreshed on a daily basis.

Behavioral observations

An ethogram and time schedule for the behavioral observations of black grouses in captivity was established and can be seen in annex I. The different behaviors were selected for the ethogram during preliminary live observing the black grouses. The observations were performed by multiple students, therefore the chance on different outcomes of observations had to be minimized. After the ethogram was established, observations were practiced by watching and observing the birds together. A guide for observing was written and is included in annex II. By practicing together and making a guide for observing, the inter-observer reliability was kept as high as possible.

The behaviors in the ethogram are classified as states or short events. States last an appreciable time and events are instantaneous (Altmann, 1974). The amount of time spent in each state is calculated as a percentage of the corrected total observed time. For event behaviors, the number of times the behavior is performed per hour is calculated. Preening and foraging are event behaviors of considerable length, and they are better shown as percentage, therefore another chart was made for these behaviors.

The four black grouses were filmed with two cameras in their cage for two and a half hours per day. When filming, there were no people present, for it was noticed – during the live observations – that the presence of humans influenced the behavior of the birds. The behavior of the birds was scored using focal sampling. A combination of continuous recording and one-zero sampling was used. For every 30 seconds it was noted which behaviors the focal bird displayed in that time, and every bird was observed individually for approximately two hours a day. However, the camera's did not cover the whole cage, therefore sometimes an individual could not be seen on video. If the bird did not show at all, the 30 seconds were noted as invisible. The data was then corrected by not counting these invisible 30 seconds as observed time. If a bird was partially invisible, the behavioral data was corrected by counting only 15 seconds. The first half hour of the films was not scored as the placing of the cameras disturbed the birds and their behavior, whereas the goal of this study was to score normally occurring behavior.

GPS equipment

The GPS transmitter (Path Track Limited) used for this project is provided with solar panels that charge the battery. In order to collect data without disturbing or catching the birds, the data points can be transmitted through the GSM network and downloaded wireless on the computer. The transmitter should work for approximately 12 months after configuration and activation. The GPS transmitter weighs 21 – 21,5 grams including harness, which is below 3% of the body weight of the female black grouses. The transmitter was painted black, so the birds would stay camouflaged and would not stand out for predators. The size of the transmitter is 3 x 5 cm and the antenna is 3 cm long. In order to prevent feather damage and covering of the solar panels by feathers, the transmitter was glued to a foam pad, which lay between the feathers and the transmitter.

Figure 2. Greyhen 2 in the soft release cage with her transmitter attached via a harness.

The GPS-transmitters are attached to the birds with a harness made out of 6,5 mm thick teflon tape. The harness was closed with two aluminum clamps. These materials came from the "telemetry logger mounting set" from ecotone (Ecotone, 2016). A more detailed description of how the transmitter was attached to the grouses can be found in annex III.

Influence transmitter

The black grouses have to be caught and handled in order to attach the transmitter on them. Together with other measurements and examination, the catching and handling takes approximately 20 minutes per bird. Considering the black grouse is extremely shy of humans, catching and handling will cause temporary physical and psychological stress and discomfort. According to the "Instantie voor Dierenwelzijn" (IVD) – which surveilles and makes suggestions on the welfare of laboratory animals at the UMC and Utrecht University – this causes mild discomfort to the black grouse. After the transmitter is attached and the birds are released in their cage again, they might experience fear of their transmitter for a longer time. This could cause moderate discomfort to the birds.

To investigate whether the transmitter has an influence on the behavior of the black grouses, the birds were observed before and after the attachment of the transmitter. Before attaching the transmitter, the behavior of undisturbed captive black grouse was filmed for ten days. The birds were observed from the videos, using the established ethogram. On May 25, 2016, the four grouses were equipped with a GPS transmitter (also see figure 2). The behavior of the birds carrying a transmitter was filmed the first ten days. The first eight days were observed in order to determine a pattern in the course of the behavior after attaching the transmitter.

The behavioral data were then processed in Excel. The percentages of state behaviors were calculated, and events were calculated in number of times per hour. To calculate the percentage of a state behavior, the number of times the behavior was tallied was divided by two, in order to calculate the time (in half minutes) the behavior was performed. Then the total performed time was divided by the corrected total observed time and multiplied by 100. The total corrected observed time is the time the bird was visible, so the time watched minus the seconds the bird was not visible. To calculate the number of times an event behavior is performed per hour, the number of times the behavior was tallied is divided by the corrected total observed time (minutes) and multiplied by 60.

Behavioral data do usually not follow a normal distribution, and are therefore analyzed using non-parametric tests, such as the Mann-Whitney-U test to compare experimental groups. However, the sample size in this study is extremely small, and the analyses might provide the wrong security of a significance values, which in fact can lead to Type I (the null hypothesis is true, but rejected by the test) and II (the null hypothesis is false, but not rejected by the test) errors, resulting in wrong conclusions. Therefore, the results are presented graphically.

Before the birds were observed, they were moved from the breeding cage to the soft-release cage. Therefore the birds were captured from their old cage, enabling us to examine the birds before the start of the experiment. The birds were weighed and their pectoral muscle mass was palpated. This was done again the moment before the birds received their transmitter and three weeks after the attachment of the transmitter. At the same three moments, multiple pictures of the birds were taken. One showing the wings, a picture showing the tail and one picture of the head. This way, the influence of the transmitter on the health, weight and feather conditions on the black grouses was investigated.

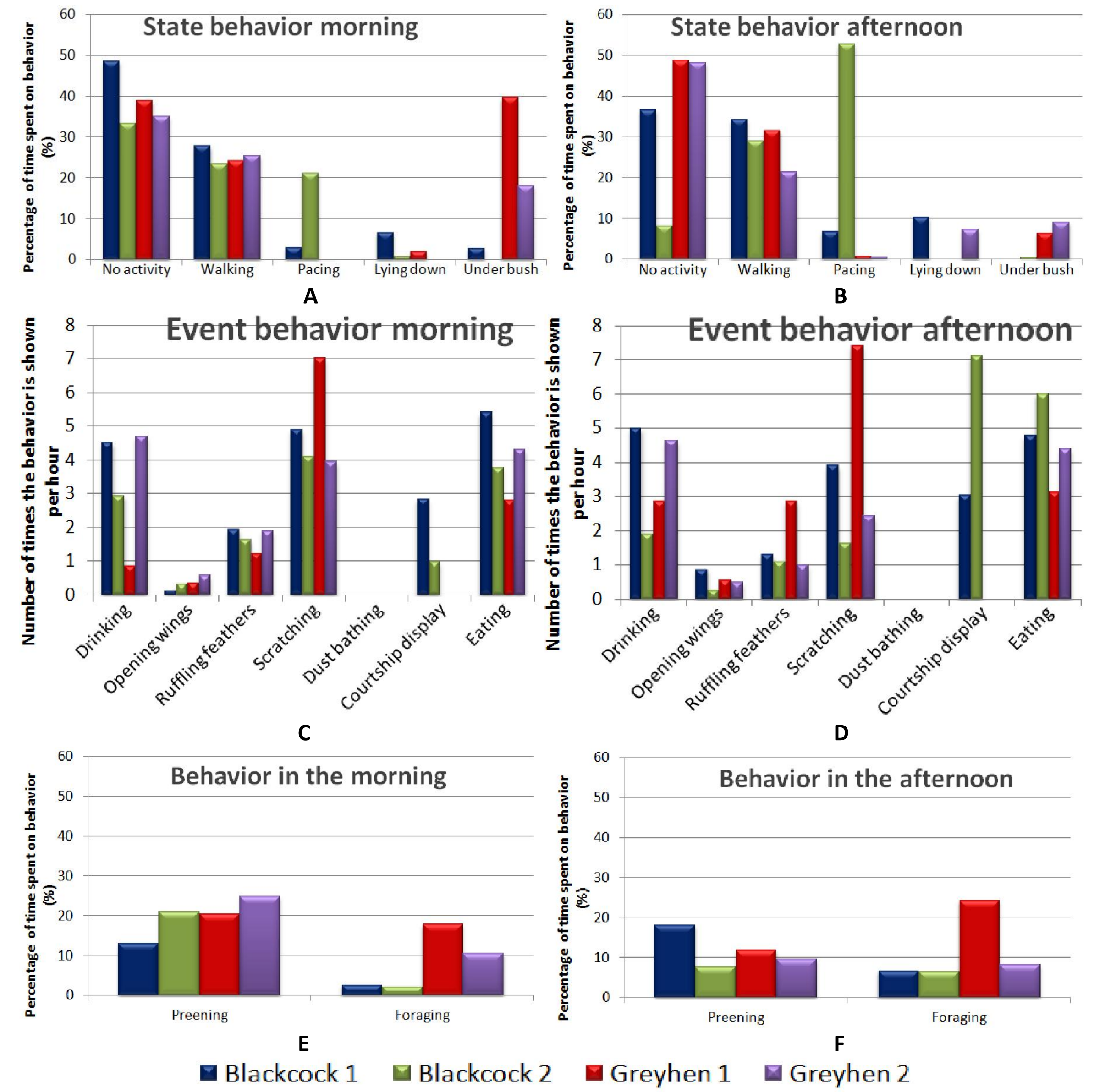

Figure 3. The normal behavior patterns of 4 black grouses, two blackcocks and two greyhens. The graphs are showing state behaviour in the morning (A) and afternoon (B), event behaviour in the morning (C) and afternoon (D) and preening and foraging in the morning (E) and afternoon (F). The state and foraging and preening graphs are showing the percentage of observed time spent on each behaviour. The event graphs show the number of times each behaviour is performed per hour.

Results

Behavioral observations

In total, 30,5 hours of film were recorded and scanned; 15 hours without the GPS-tag and 15,5 hours with the GPS-tag. Because the cameras did not cover the whole cage and the birds were not always in sight, the time the birds were observed was 10 hours without the GPS-tag and 6 hours with the GPS-tag.

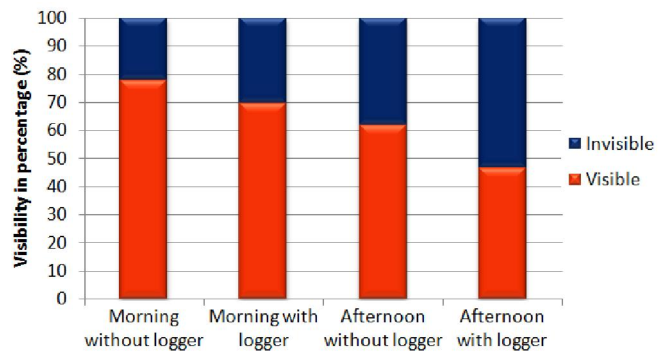

Figure 4. The average percentage of (in)visibility of the grouses with and without the logger, during the morning and during the afternoon.

The data showed that there was a difference in behavior in the morning and the afternoon. For example, the birds were more visible in the morning than in the afternoon, see figure 4. Therefore the graphs in this report are split up in graphs showing behavior in the morning and graphs showing behavior in the afternoon.

Normal behavior

The most frequently performed behaviors are shown in figure 3. Figure 3 also shows the differences in behavior between male (blackcock 1 & 2) and female (greyhen 1 & 2) black grouses. Females spent more time on foraging whereas males ate more frequently from the food bowl. Females spent more of their time hiding under a bush. Males showed courtship behavior, while females did not. Besides these differences, the average behavior of the two males and two females was quite similar; therefore the behaviors could be summarized when looking at the effects of the transmitter. However, birds differed individually with respect to some behaviors. For example blackcock 2 was pacing a lot during the study, while the other birds almost never paced and greyhen 1 scratched a lot during the observations.

The average behavior of all four birds in the morning and afternoon can best be seen in figure 6, by looking at the blue bars. Diurnal variation is mostly seen in preening (19% in the morning; 12% in the afternoon), under bush (17% in the morning; 4% in the afternoon) and foraging (9 times per hour in the morning; 13 times per hour in the afternoon). Furthermore, figure 4 shows that the birds were considerably more visible during the morning than during the afternoon, indicating a diurnal variation in behavior.

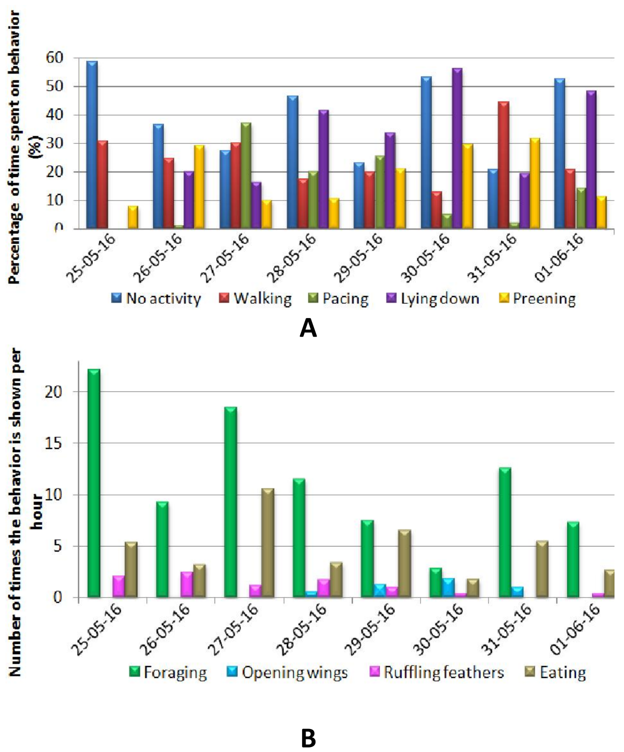

Figure 5. The course of the average behaviour of all grouses after receiving the logger on may 25, 2016. State behaviour is shown in percentage of time per day (A) and event behaviour is shown in number of times per hour (B).

Behavior after the attachment of a transmitter

After handling the birds to attach the transmitter, the birds were scared and barely came out of cover on the first day. Blackcock 1 and greyhen 1 were in sight for three quarters of an hour and blackcock 2 and greyhen 2 only showed themselves for two minutes. In the time the birds did show themselves, they were mainly just standing still (no activity) or foraging. Greyhen 1 spent four minutes on pecking the transmitter and harness the first day. The second day no bird touched the transmitter or the harness, and only on the seventh day, blackcock 2 pecked the transmitter once. So seemingly, the transmitter did not cause the grouses to obsessively peck at the harness or the transmitter.

The course of the behavior over the first eight days after attaching the transmitter is shown in figure 5. The average behavior of all four grouses is shown, for each filmed day. It can be seen that the birds did not spent a lot of time on preening the first day, in fact the first day the least amount of time was spent on preening. To compare the behavior before and after the attachment of the transmitter, the average behavior of all birds – before and after receiving the transmitter – is shown side by side in the graphs of figure 6. Figure 6 shows that in total, the amount of time spent on preening did increase, especially in the afternoon. This could be an effect of the harness and sender. A pattern in the course of time spent on preening the first 8 days cannot be found, since figure 5 shows peaks and dips over the whole time. The first days, the greyhens were not flying away when someone approached their cage, while before the attachment of the transmitter, they always stressfully flew away (the blackcocks were not able to fly, due to their damaged feathers). After four days, the hens started trying to fly and after two weeks they were able to fly through the cage again.

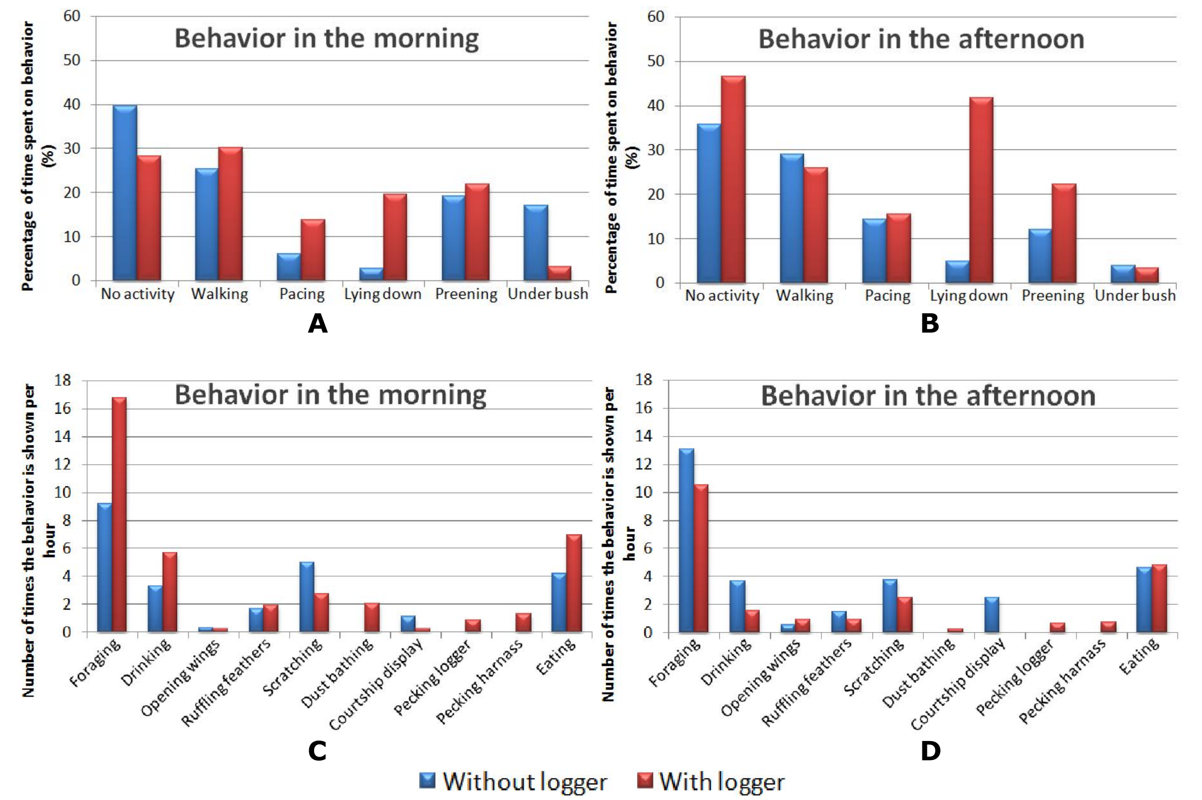

Figure 6. The difference in behaviour, before and after the attachment of the logger. The average state behaviour of four black grouses – with and without logger – in the morning (A) and afternoon (B) are shown. The average event behaviour in the morning (C) and afternoon (D) are also shown.

There does seem to be a slight decrease of the behavior 'ruffling feathers' over the course of eight days. Figure 5 shows that the first days they were ruffling feathers for two times an hour, then it decreases to one time per hour and the last days almost zero times per hour. So it seems like the transmitter made the birds ruffling their feathers more at the beginning. By looking at figure 6, it can be stated that showing the behavior 'ruffling feathers' did increase a little bit in the morning, however in the afternoon the birds were ruffling their feathers way less often. Therefore it is difficult to ascertain the effect of a transmitter on the amount of 'ruffling feathers' by the birds.

The birds were still foraging and eating out of the food bowl as much as they were without the transmitter, which is a positive sign. They were however lying down more and the birds started dust bathing, while they had not done that before. This could also be a small effect of the sender. The blackcocks stopped with showing courtship display. The other behaviors did not change much. The grouses did not perform any extreme behaviors due to the attachment of the transmitter. The males were pacing during the observations, but they already performed this behavior before the transmitter was attached.

A

B

Figure 7. The right wing of blackcock 1 (A) and the left wing of greyhen 1 (B).

Health, feather condition and weight

The results of the measurements taken before the movement, before attaching the transmitter and after attaching the transmitter are shown in table 1. Seven weeks after moving to the soft-release cage, three birds lost weight and greyhen 2 gained some weight. Then, three weeks after attaching transmitter, the same three birds gained weight, and greyhen 2 lost some weight.

Table 1. The weight and size of pectoral muscle mass of the black grouses during the research. The size of the pectoral muscle mass was scored on a scale from 0 to 3: 0 - Sternum sharp, muscle depressed; 1 - Sternum easy to distinguish, muscle neither depressed, sharp but not sharp nor rounded; 2 - Sternum still distinguishable, muscle slightly rounded; 3 - Sternum difficult to distinguish, muscle rounded (full) (Barlein, 1995).

Bird	Before the movement to the soft-release cage		Moment before attachment transmitter		Three weeks after attaching the transmitter	
Date	April 4, 2016		May 24, 2016		June 14, 2016	
	Weight (g)	Palpation pectoral muscle mass	Weight (g)	Palpation pectoral muscle mass	Weight (g)	Palpation pectoral muscle mass
Blackcock 1	1046	2,5	950	2	967	2,5
Blackcock 2	1067	3	998	2,5	1046	2
Greyhen 1	820	2,5	752	2,5	771	2,5
Greyhen 2	817	3	832	2,5	799	2,5

When capturing the birds for the first time, it appeared that the feathers of the blackcocks were in a bad condition (figure 7). This remained so during the whole research. At the second measuring moment their feather condition was even worse than before. At the end of this research, the grouses were molting and new feathers had grown. The greyhens had better feather conditions, as seen on figure 7. At the second measuring moment their feather condition seemed a little bit worse. But at the last photo's taken, their feathers looked good again so the second measurement was probably influenced by catching the birds in wet grass. Greyhen 2 was first bald and later wounded on her head. She did recover from her wound during the last three weeks of the research. From the examination of all birds, it appeared that the transmitter and the harness did not cause damage to the feathers or the skin. Annex IV contains photos of all birds before the movement, after the movement and after the attachment of the transmitter.

Discussion

Behavioral observations

This study is one of the few that investigated the effects of transmitter attachment on behavior and physiology of black grouse. Despite the low sample size, the observations serve to get an impression of the behavior of black grouse in captivity. For instance, they suggest some sex-specific differences, which should be taken into account when housing these birds.

Normal behavior

Females are foraging much more than male birds and this difference in foraging behavior between male and female could mean that females adapt easier in the field once they are released. Moreover, greyhens hide under bushes more and they are better camouflaged than the blackcocks. This implies that females have a higher chance of surviving once released into the park.

The increase in foraging in the afternoon does not seem to be in line with the foraging behavior of other bird species. The regular foraging pattern of birds is heavy feeding in the morning, light feeding in the early afternoon and moderate heavy in the late afternoon (Bednekoff, 1994). However, the normal foraging pattern of black grouses is not yet studied in detail and the early morning peak might have been missed during the observations in this study.

This research suggests black grouses mostly hide under bushes in the morning. It is however likely, that the birds were hiding under bushes in the afternoon, as well, which would explain the large amount of time the birds were invisible (on the video recordings) in the afternoon. Thus, no strong conclusions can be drawn about the time spent in cover.

Our study revealed that the blackcocks, mostly blackcock 2, were pacing. Pacing is a stereotypical behavior and might reflect an impaired welfare of the individual. Stereotypical behavior of animals is the result of frustration (Ödberg, 1978). When an animal cannot perform appetitive behavior or when consummatory behavior occurs without showing the appetitive behavior (for example cows eating without grazing), this leaves animals in states of high motivations. When this high state of motivation sustains, it can lead to frustration related stress (Mason et al., 2001). The frustration of appetitive behavior can lead the animal into a cycle of repetitive and stereotyped behavior, like pacing (Hughes et al., 1988; Wood-Gush et al. 1989). In chickens it is known that food deprived chicks, food frustrated hens and laying hens that cannot nest, show pacing behavior (Campbell et al., 1966; Muir et al., 1998; Duncan et al., 1971). When male birds are prevented from reaching a female, stereotypic behavior can be developed (Ödberg, 1978). The blackcocks in this study were showing courtship behavior, which is good because it is part of their natural behavior pattern (van der Ziel et al., 2004). However in nature, they would go lekking, i.e. assemble with other males at certain lek spots to attract females. Blackcocks in captivity are not able to go lekking and this constraint might cause frustration causing the cocks to pace.

Another explanation could be the unlimited food supply causing frustration of the birds. Food out of the food bowl is nutritious and birds do not need to forage to satisfy their hunger. However, foraging is an inherent need of animals, and many species prefer to work for their food (Lindqvist et al., 2008). Our results showed that the male birds were indeed not foraging as much as the females.

It is difficult to ascertain the cause of the pacing behavior. Maybe, the birds were only pacing because of the disturbance by humans and they wanted to escape but could not. It can, however, be concluded that the blackcocks are frustrated: some goal cannot be reached, some homeostasis is disrupted or some tendency is being thwarted (Mason, 2006).

Behavior after the attachment of a transmitter
The first hours after the attachment of the transmitter, the grouses stayed hidden for a very long time. This indicates that catching and handling was highly stressful for them and that they were still fearful hours after the researchers left. Therefore, it was very positive that when they did show themselves, they were foraging and eating and not showing any abnormal or extreme behaviors, like walking backwards, trying to remove the harness or even showing aggression towards each other. It is remarkable that the grouses were not preening a lot the first day, since catching and handling disturbed their feathers quite a lot. It is, of course, possible that the birds were preening a lot, just not in front of the camera, since they had stayed hidden for so long. Another possibility is that they had to recover from the stress that they have experienced first, but then one would not expect the birds to eat and forage as much as they did.

The percentage of the time the birds were visible slightly decreased. This suggests that the grouses became more fearful after they received the transmitter. Being fearful more often is also an impairment of welfare. It is, however, difficult to estimate whether the grouses were really more fearful than before, more observations would be necessary.

The blackcocks did not display any courtship behavior anymore after they received their transmitter, which might be due to a negative effect of the sender but also merely due to the time of year. The peak of lekking starts late April and ends in May (Starling, 1992), so courtship display could be less shown in end May/June. However blackcocks should be lekking and thus showing courtship display throughout the whole year. It could be of influence that the birds used in this study were only yearlings and thus not that experienced with showing courtship display. Another possibility is that the blackcocks were only showing courtship activity after the sunset now that the mating season is over, while the grouses were only filmed in the mornings and afternoons. Nevertheless, the consequences of a transmitter on the courtship display behavior of blackcocks will have to be further examined, because it is not effective to reintroduce a blackcock that will not reproduce.

The transmitter also seemed to influence the stereotypical behavior, as the amount of time spent on pacing increased. This indicates that the transmitter has a negative effect on the welfare of the black grouses.

Health, feather condition and weight
The feathers of the male grouses were damaged prior to attaching the transmitter, because the birds flew against the netting of their homecage whenever unfamiliar people or predators approached. As the birds could not fly due to the damaged feathers, we could not examine the influence of the transmitter on the flying behavior of the male birds. Nevertheless, the examination of the back and wing feathers pre-and post attachment showed that the transmitter did not influence the feather condition negatively in any of the birds. The birds were molting, which is a good sign, because it shows a bird can enter their molt, while wearing a transmitter.

The initial weight loss of three of the grouses can be related to their movement to the soft-release cage. The translocation was stressful and in the beginning the birds were not used to the cage yet, which might have caused them to eat less. Furthermore, it was the mating season so maybe there was less focus on eating and more focus on courtship display. After the second measuring moment, the birds were already used to the cage, they now had to get used to the transmitter. However, in

May and June, there were more insects for the grouses to eat and the mating season was over. The results also showed that the grouses were eating and foraging more in this period. Most importantly, the transmitter did not prevent the grouses from gaining weight in this last period.

Ethical considerations

The black grouse is a sensitive bird species and reintroducing it, with or without transmitter, is quite a burdensome experience for the individual birds. In order to estimate the effect of the introduction and the transmitter on black grouses, animal welfare must first be defined. A former definition of animal welfare was based on the exception of negative attributes. It was suggested by the Brambell Committee (1965) that animal welfare was preserved if animals were kept free from:

1. Hunger, thirst or incorrect food;
2. Thermal and physical discomfort;
3. Injuries or diseases;
4. Fear and chronic stress;
5. Free to display normal, species-specific behavioral patterns.

However, animals are able to interact with and adapt to their environments. For example, hunger is a negative feeling, but if a hungry animal has access to feed and can eat enough, animal welfare is not impaired. A biological based animal welfare concept therefore includes the ability to react and adapt to the five aspects of animal welfare (Ohl et al., 2012). After the new insights, the five freedoms from the Brambell Committee were adjusted (Ohl et al., 2012): An animal is in a positive welfare state when it has the freedom to adequately react to:

1. Hunger, thirst or incorrect food;
2. Thermal and physical discomfort;
3. Injuries or diseases;
4. Fear and chronic stress;
5. Free to display normal, species-specific behavioral patterns that allow the animal to adapt to the demands of the prevailing environmental circumstances and enable it to reach a state that it perceives as positive.

The latter animal welfare concept will be used to assess the welfare of the black grouses in this project.

Reintroduction

The reintroduction of black grouses in the Hoge Veluwe comprises two or three stages. Each black grouse starts its life in the breeding cage, some individuals then move to a soft release cage, others stay in the breeding cage and once they are old enough they will be released into the park of the NPDHV. Regarding the five freedoms, the welfare of the grouses in the first two stages is not so bad. They have the freedom to adequately react to hunger and thirst, thermal and fysical discomfort, injuries and diseases and they are free to display normal and most species-specific behavioral patterns. However, there are some points of attention. As mentioned before, the grouses are very shy of humans and they are certainly not domesticated. So whenever people (other than the animal caretakers) walk by or when predators are nearby, the birds experience a lot of stress and fly through the cage against the netting of the cage. This causes severe feather damage, as seen on the male birds of this research, and even injury to the head, as seen with greyhen 2. These injuries could be prevented using another, softer, material to seal the walls and ceiling of the cage.

The birds were also pacing, which is a stereotypical behavior indicating frustration related stress and an impaired welfare. Reasons could be the restriction from some species-specific behaviors, like lekking or migrating over long distances, which leads to impaired welfare (see point 5 above). However, these restraints are unlikely to be resolved for black grouses in captivity.

Once the birds are released into the park of the NPDHV there are some improvements and several diminishments for the welfare of the black grouse. Of course, being free is an improvement in welfare. The free grouses can migrate over great distances now and males can go lekking. The birds can choose whether to be together or on their own (which wild black grouses usually are). So their freedom to display natural behavior patterns increases greatly. In captivity, the birds are provided with high nutritious foods and have unlimited access to it. Some natural foods were given, like branches of larchs, and in the soft release cage the birds could also forage for insects. However, the larchs and insects seemed more like an enrichment for the birds, especially for the males, but were not enough to satisfy their hunger, as they ate mostly from the provided feed. Therefore, in the park they might not be able to find enough food and cannot react adequately to their hunger. Moreover, it is not certain whether the captive reared grouses in this project can adequately react to their fear of predators. During the observations, it was seen that they did react to goshawks flying over, by hiding under bushes or displaying freezing behavior. Yet, the number of surviving grouses after release suggests they are not able to adequately react and hide well or fast enough. Training of anti-predator behavior in captivity might be a way to better prepare the grouse for a life in the wild.

GPS transmitter

After observing the tagged birds, an impression of the welfare – with regard to animal welfare concept of the Veterinary faculty – of black grouses wearing transmitters can be obtained. With a transmitter, the birds were able to react adequately to their hunger, since they were eating as much or even more. In the beginning, the transmitter might have caused some mild physical discomfort. Yet, the birds did not show many signs of physical discomfort, other than pecking the transmitter and harness a few times on the first day. They did seem to refrain from flying on the first few days, as they did not fly through the cage when the camera was set up for observations. But after habituation occurred, the birds did fly again with transmitter. The transmitter did not cause any injuries or diseases on the birds. Catching and handling the grouses is probably the most stressful part of the whole process and causes moderate discomfort for the black grouses. All birds were showing mild signs of stress when handled during the attachment of the transmitter, such as increased breathing. However, they quickly recovered after release.

The stereotypical behavior ‘pacing’, increased a little bit after the transmitter was attached. This might be sign of impaired welfare due to the transmitter. However, as this stereotypy was seen already before the attachment, it is unclear what impact the transmitter itself had.

Overall, the effects of wearing a transmitter on the welfare of black grouses are quite small. It is the handling and attachment of the transmitter that has a measurable negative effect on the welfare of the grouses. However, attaching the transmitter is a one-time event and only lasts for about 20 minutes, and the birds seem to cope and recover over time, as seen from the behavioral and physiological measurements.

IUCN

The ICUN Guidelines on reintroduction programs also contains a paragraph named ‘welfare’. It states that “Every effort should be made to reduce stress or suffering.” In order to comply with that rule, some changes have to be made. For instance, the cages should be adjusted so birds cannot hurt themselves when flying against it. Also, it would be better if the birds in captivity were not provided with nutritious feed and only fed with feed derived from the park. This way they would already be used to this diet and the released grouses would most likely suffer less from hunger. If the reintroduction is really conducted conform the IUCN guidelines, it can be questioned whether it is respectable to study the released birds with GPS tracking since this causes a short time of moderate discomfort and stress for the grouses. But the IUCN also finds that the success of the introduction should be monitored and a lot of information can be gathered from tracking the released birds with GPS transmitters.

DEC

In the DEC application, it was estimated that the discomfort of the grouses in this research would be mild. It was estimated that the handling, measuring and attaching the sender would take 15 minutes and would cause a mild distress of the birds. However, the birds were extremely fearful and started panting after a while. After handling them, they stayed away hidden for quite some time, indicating that the birds were still stressed after the research team left. It was estimated in the application that the birds would experience mild discomfort from the transmitter and that the grouses would get used to the sender in one day. This study revealed that the birds indeed needed less than a day to get used to the sender, which is positive. So overall it seems, that the capture and handling causes more discomfort to the birds than the transmitter itself. Therefore, the procedures should be carried out as quick as possible and only with experienced people, while the bird should be closely monitored.

Hypothesis

The results of this study are not strong enough to reject the null or alternative hypotheses (see limitations and future recommendations). However, the research does reveal that the transmitter did not markedly influence the health, feather condition and weight of the four black grouses. It is difficult to estimate the influence of the transmitter on the behavior of the grouses, but it seemed to have some effect on preening, pacing and courtship display.

Limitations and future recommendations

Due to circumstances, there were only four birds available for the observations. This is a very small sample size, and hardly represents the whole population from the park. Also, during the first live observations it turned out, that the birds did not show their normal behavior patterns, even though the observations were made from a tent, hiding the observers. Therefore it was chosen to use video recordings. Unfortunately, all the live observation material could not be used for this study and so there were less observations of normal behavior. It is recommended to increase the sample size, so the results can be analyzed statistically. Nevertheless, the results of this study can be taken as a first indication.

In a subsequent study, it would be better to work with a cross-over design where birds with and without transmitters (control group) are observed at the same time. In this study, normal behavior was observed from mid April till May, while the observations of birds with transmitters happened from end May till the beginning of June. The seasonal differences might have influenced the behavior. For example, courtship behavior was not shown anymore after the birds received their transmitter, but lekking activity of black grouses reaches its peak late April and May (Starling, 1992) and the birds are yearlings. So maybe the birds would not be displaying courtship behavior in late May without transmitters, too. Therefore, the influence of a transmitter on courtship display could not be clearly determined in this study. Another behavior influenced by seasonal differences could be foraging, since the number of insects increased in time, during this study. Consequently, the grouses were foraging more and this study does not reveal whether the birds would have foraged even more without the transmitter.

Despite these limitations, this study conducted qualitative analyses for the rejection of the transmitter and for the manner of attaching the transmitter. If one moment of severe discomfort occurred, the protocol or the way the transmitter was attached, would have been changed. Fortunately, the black grouses used for this investigation did not show any signs of severe discomfort even though the attachment of the transmitter leaves some area for improvement. Attaching the transmitter on the birds took quite a long time and since handling the birds costs them moderate discomfort, a faster attachment has to be developed.

Conclusion

This study gives a first impression of the influence of the attachment of a GPS transmitter on black grouses using a body harness. The harness and sender did not seem to have any influence on the health, weight or feather condition of the grouses. In terms of behavior, there were some small changes. The most important were the increase in pacing, the decrease in courtship behavior and that the birds had to get used to the transmitter before they were able to fly again. The increase in pacing indicates a negative effect of the transmitter on the welfare of black grouses. Catching, handling and attaching the sender caused the most discomfort for the birds, therefore this should be performed as fast as possible. Further research with a cross-over design and a bigger sample size is necessary to determine the true effect of a transmitter on black grouses.

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Annex I The ethogram

Time	Invisible	Partial invisible	Under bush	Freezing	No acivity	Walking	Running	Lying down	In bush	Foraging	Eating	Drinking	Opening wings	Ruffling feathers	Stretching	Preening	Scratching	Pacing	Flying	Roosting	Dust bathing	Threatening	Chasing	Pecking	Courtship	Pecking logger	Pecking harness	Prostration
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Annex II Guide for observing the birds

Birds
Blackcock 1: The cock with the purple colored ring
Blackcock 2: The cock with no colored ring
Greyhen 1: The hen with the purple colored ring
Greyhen 2: The hen with no colored ring

Method

- Note every behavior that is performed in each half minute on the ethogram;
- Behaviors are only noted once per half minute;
- When a bird is still performing a behavior when the next half minute starts, this behavior is also tallied in the next half minute;
- The first half hour of the video material is not observed.

Behaviors

- No activity: no other behavior is performed in the entire half minute;
- When a bird is roosting, flying behavior is not tallied;
- If the wings open while the bird is stretching or ruffling its feathers, opening wings is not tallied;
- Flapping the wings counts as opening wings;
- Tally 'invisible' when the bird was invisible for the entire half minute;
- Tally 'partial invisible' when the bird was not visible for a part of the half minute. When a bird walks behind a bush and could not have performed any other behavior in the time it was not visible, 'partial invisible' should not be tallied;
- Pacing: a bird is walking the same route three times in a row or a bird is walking back and forth within a short distance;
- Foraging: eating from the ground, bushes or form the walls of the cage;
- Eating: eating out of the food bowl;
- Freezing: a stressful event occurred and at least three of the four birds are standing completely still. Often the birds have a stretched neck, while they are freezing as seen in figure 1.

Fig. 1 Three black grouses are freezing and stretching their neck.

Annex III Guide for attaching the transmitter

This step-by-step guide shows how the transmitter was attached to the black grouses in this study. The guide was made using a silkie (*Gallus gallus domesticus brisson*).

1. Place the transmitter on the back of the bird with the solar panels directed upwards and the antenna directed causally.

2. Put both cords over the head of the bird.

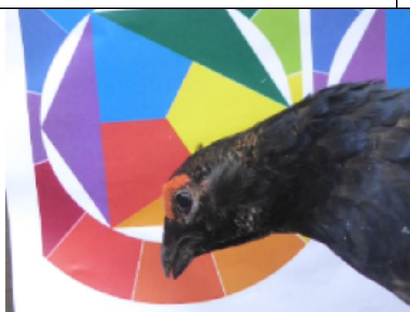
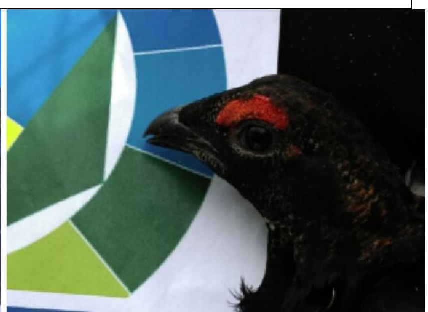
3. Then place the loops around the wings, one by one.

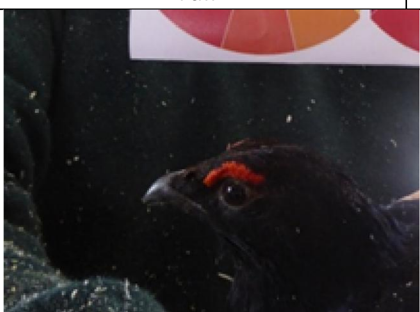
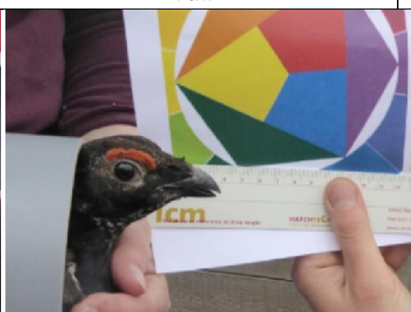
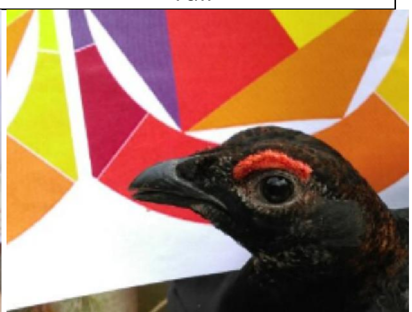
4. Tighten the cord at the chest, so the loops around the wings become smaller. The small ring from the harness has to be positioned caudoventrally on the chest.

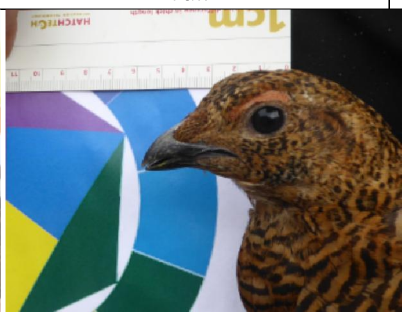
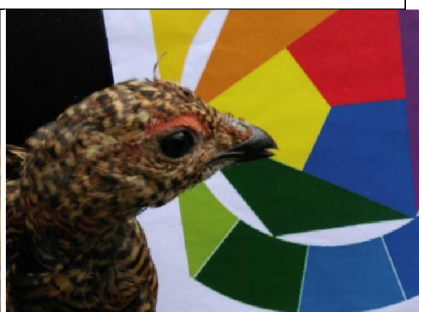
5. Place the loose ends around the head, to the transmitter on the back.
6. Then pull the cords through the openings on the transmitter.
7. Ensure the cords are tight enough, but not too tight. Two fingers must fit under the cord.
8. Pull the end of the cords through the aluminium ring and press the ring with a plier so the cords are locked into place.

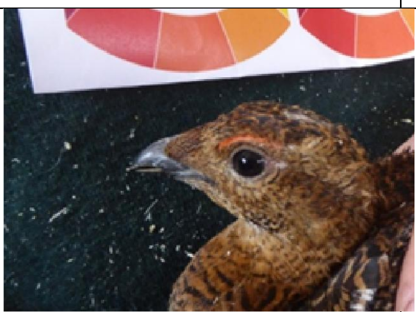
9. This is what the transmitter should look like on a black grouse.

Annex IV Photos of the birds during the study

Blackcock 1		
Before the movement	**After the movement**	**After the attachment of the transmitter**
 Left wing	 Left wing	 Left wing
 Right wing	 Right wing	- Right wing
 Tail	 Tail	 Tail
 Head	 Head	 Head

Blackcock 2		
Before the movement	**After the movement**	**After the attachment of the transmitter**
 Left wing	 Left wing	 Left wing
 Right wing	 Right wing	- Right wing
 Tail	 Tail	 Tail
 Head	 Head	 Head

Greyhen 1		
Before the movement	**After the movement**	**After the attachment of the transmitter**
 Left wing	 Left wing	- Left wing
 Right wing	 Right wing	 Right wing
 Tail	 Tail	- Tail
 Head	 Head	 Head

Greyhen 2		
Before the movement	**After the movement**	**After the attachment of the transmitter**
 Left wing	 Left wing	 Left wing
 Right wing	 Right wing	- Right wing
 Tail	 Tail	- Tail
 Head	 Head	 Head