Bachelor final thesis report

Effect of Low Pathogenic Avian Influenza on winter movement and fuelling rate in Bewick's swans (*Cygnus colombianus bewickii*)

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Photograph front page: O. de Vries

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Acknowledgment

In front of you lays our final thesis report for our bachelor in Animal Management with the major Wildlife Management. We conducted the research for the Netherlands Institute of Ecology (NIOO).

We want to use this page to show our gratitude to the people who have guided us or helped us during this final phase of our study. First of all we like to thank Hans Bezuijen and Theo Meijer of the van Hall Institute, who guided us during the whole process and gave comments on our report.

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Leeuwarden, April 29th 2010,

Andrea Vos
Wouter Wietses

Summary

The migration ecology of waterfowl, is considered to be a major factor in the transmission and the geographical spreading of Low Pathogenic Avian Influenza (LPAI) viruses. However not much is known about the pathological effects of a LPAI infection on wild waterfowl. In laboratorial studies only mild symptoms were observed of the virus on its host. Mild symptoms observed in a laboratory can however have severe impacts on an individuals health in the wild. A previous research showed that wild Bewick's swans infected with a LPAI virus, showed negative effects by fuelling and feeding on reduced rates and having a delayed migration. These findings were based on a small sample size, namely two naturally infected Bewick's swans. This study is a continuation of these findings, by examining the consequences of an Avian Influenza infection on the dispersal and fuelling rate of Bewick's swans.

To answer these questions, data of the last five years were combined. Bewick's swans were caught using cannon netting in the winters between 2005 and 2009. The swans were discriminated in four research groups, namely experimentally infected (swans that were inoculated with an Avian Influenza virus at capture), naturally infected (swans that had an Avian Influenza infection at capture), Phosphate Buffered Saline (PBS)(swans that were inoculated with PBS solution at capture) and control (swans that were not assigned to the other research groups). Some Bewick's swans were fitted with a GPS neck-collar. The data for examining the dispersal included data points of individual swans obtained from GPS tracking and resightings of (mostly) amateur bird watchers. Data of the fuelling rate was obtained by examining the Abdominal Profile Index (API) of Bewick's swans. An API represents the shape of the belly between the tail and legs. The fuelling rate was expressed as the change in visually scored API's per day. The dispersal was examined on day 2, 5, 10, 14 and 30 after capture. A condition index was calculated to account for body mass by structural body size and body mass which could be accounted for by extra energy stores or fat.

No significant differences were found between the four treatment groups in condition index. There was no significant difference in dispersal between the treatment groups on all examined days. The fuelling rate didn't show a significant difference between the research groups as well. As there were no differences observed between the research groups, we concluded that an infection with an Low Pathogenic Avian Influenza virus probably doesn't have long-term effects on Bewick's swans.

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1. Introduction

1.1 Background

There are three types of Influenza viruses (*Orthomyxoviridae*) by which humans can be infected, namely type A, B and C, of which type A is the most severe and type C the least (Centers for Disease Control and Prevention 2005). Influenza A is further classified by subtypes. The subtypes are based on the surface proteins *hemagglutin* (HA) and *neuraminidase* (NA). Today there are 16 different HA proteins (H1 - H16) recognized and 9 different NA proteins (N1 - N9) (Centers for Disease Control and Prevention 2005). Avian Influenza viruses have been recorded in at least 105 wild bird species in 26 different families (e.g. Stallknecht & Shane 1988, Webster *et al.* 1992, Olsen *et al.* 2006). The *Anseriformes* (ducks, swans and geese) and *Charadriiformes* (gulls, terns and waders) are considered to be the major natural hosts of most Avian Influenza viruses (Webster *et al.* 1992, Olsen *et al.* 2006). Avian Influenza viruses infect the cells of the intestinal tract and are excreted in high concentrations in bird faeces (Slemons & Easterday 1978, Webster *et al.* 1978). The relative high prevalence of Avian Influenza viruses in wild birds living in an aquatic environment may be due to the relatively long survival of the Avian Influenza viruses in aquatic environments, which facilitate an efficient faecal-oral transmission (Webster *et al.* 1992).

The infection of Avian Influenza viruses can cause disease symptoms in domestic poultry, depending whether it is a Low Pathogenic Avian Influenza (LPAI, causes mild disease symptoms) or High Pathogenic Avian Influenza (HPAI, severe illness and can cause death). It is also known that LPAI viruses can mutate into HPAI viruses (Centers for Disease Control and Prevention 2005). The mutation of Influenza A virus into a subtype which can infect humans and causes disease symptoms, is called antigenic shift. Antigenic shift can happen through animal-human transmission of Influenza A viruses or through mixing of Influenza A human subtype with an Influenza A animal subtype. The subtypes H1N1, H1N2, H3N2 and H5N1 are currently circulating among humans. Transmission, from birds to mammalian species, of different Influenza A subtypes are recorded at live bird markets where there were multiple bird species and mammalian species in close contact (Webster *et al.* 1989). The distribution of Avian Influenza is therefore a concern to human and animal health and to the world economy (WHO/Europe 2009).

An individual can react in two different ways on a virus infection, the pathogens can either be resisted or tolerated. Both strategies are thought to come at a high energetic cost (Behnke *et al.* 1992, Read *et al.* 2008, Råberg *et al.* 2009). This could mean that an individual needs to allocate resources to its immune system at the cost of (long-term) fitness investments such as migration and/or reproduction. The migration ecology of the LPAI viruses' hosts is considered to be a major factor in the transmission and the geographical spreading of Avian Influenza viruses (Olsen *et al.* 2006).

Due to the short Arctic summer, the timing of spring migration for arctic breeders, and thus the Bewick's swan, is crucial for a successful breeding season (Drent & Daan 1980, Klaassen 2003). The arctic summer contains 120 frost-free days. The breeding cycle of the Bewick's swan is 110 days (Beekman *et al.* 2002). Bewick's

Swans are therefore thought to be one of the most time-stressed birds, and capital breeding may be the only way to have a successful breeding season (Nolet 2006). Capital breeding means that the birds bring along energy stores (capital) from their wintering grounds, or along the flyway, to their breeding grounds (Drent & Daan 1980). Many researchers acknowledge the importance of post migratory residual body stores for successful breeding (e.g. Drent & Daan 1980, Ebbinga & Spaans 1995, Bêty *et al.* 2002). Due to the lack of feeding opportunities at arrival on the breeding grounds, post migratory residual body stores can enable a rapid initiation of egg laying and thus shortening the number of days needed on the breeding grounds (Klaassen 2003). Drent & Daan (1980) also mention the importance of body conditions as a predictor of breeding success.

1.2 Problem description

It was always thought that LPAI viruses didn't cause diseases in wild bird species (Webster *et al.* 1992). However most of the known research is based on experimental infection of birds in captivity rather than natural infections by wild migrating birds (Webster *et al.* 1992). Sub-clinical or mild disease symptoms observed in the laboratory may have significant ecological consequences in the field (Gils *et al.* 2007), as the health of an individual plays in important role in its geographic distribution, survival and breeding success. Bewick's swans (*Cygnus columbianus bewickii*) naturally infected with LPAI viruses, negatively effect their body mass and feeding behaviour and increase stopover time (van Gils *et al* 2007). In a study of Latorre-Margalev *et al.* (2009) a negative correlation between LPAI infection and body mass in Mallards (*Anas platyrhynchos*) was observed.

The Netherlands Institute of Ecology (NIOO) has chosen to use Bewick's swan for this research for the following six reasons: (1) the species is known to be infected with LPAI in the wild; (2) the chance that control individuals become infected is very low (because low prevalence of infection and the timeframe in which an individual can be infected within the year is very narrow); (3) it is a long distance migrant, which is the key of interest for geographic spread of LPAI; (4) marked individuals are resighted relatively easily due to it's size and conspicuousness; (5) the wild population is relatively small (<25,000 individuals); so results can be scaled up from individual to population level; (6) an initial correlation between LPAI infection and behavioural changes was observed in this species (van Gils *et al.* 2007).

Van Gils *et al.* (2007) showed that the fuelling rate of naturally infected Bewick's swans is lower and that it negatively effects the body mass. This could mean that migration (a long-term survival strategy) is pushed back in time to accumulate more energy (van Gils *et al.* 2007). The infected individuals will arrive later at the breeding grounds and therefore will have difficulties to reproduce.

1.3 Research goal

In order to gain a better understanding of the epidemiology of Avian Influenza in their natural hosts, more insight in the effects on its natural hosts should be gained.
The aim of this research is to gain insight into the effects of a LPAI infection on winter movement and the influence on the preparation for migration (especially the fuelling rate) of Bewick's swans. This is done within the framework of a larger research, which aims to interpret the potential role of wild birds in the distribution of LPAI viruses, conducted by the Netherlands Institute of Ecology (NIOO).

1.4 Research questions

1. **What is the difference in condition index at the moment of capture between infected and uninfected Bewick's swans?**

2. **What is the difference in winter movement between infected and uninfected Bewick's swans?**

3. **What is the difference in fuelling rate during the winter between infected and uninfected Bewick's swans?**

1.5 Definitions

Net dispersal	The distance between the capture location and the resighting/GPS recorded location, a certain number of days after capture.
Condition index	The residuals of body mass as a function of skeletal size (§2.7.1).
Fuelling rate	Expressed as the change in visually scored Abdominal Profile Indices per day (§2.6).
Catching site	The field where the Bewick's swans were captured on.
Catching area	The area within 15 km of the catching site.

2. Materials and methods

2.1 Study area

This research focused on the movement and the fuelling rate of Bewick's swans, while present on the wintering grounds. As mentioned below (§2.2) most of the birds of the north-western Palaearctic flyway population of Bewick's swans stay during the winter in the Netherlands. Swans were captured during winter in the Netherlands (§2.3.1). After capture, the dispersal of the individuals with neck-collars (§2.3.3), determined where and when the observations took place (§2.5 and §2.6). The fieldwork took mainly place throughout the Netherlands, as well as in some areas in Belgium, Germany and the United Kingdom.

2.2 Research population

The population of Bewick's swans (north-western Palaearctic flyway population, *C.c. bewickii*) consists of approximate 25,000 individuals (Vogelbescherming 2009). This long-distance migrant breeds on the arctic tundra in the northern part of Russia and stays during the winter in Western Europe. Most of the birds (17,000-19,000) stay during the winter in the Netherlands. The rest of the population spends their winter mainly in the United Kingdom, Germany and Ireland (Figure 1)(Vogelbescherming 2009).

Bewick's swans migrate twice a year (spring and autumn migration) and fly approximately 3000-4000 km each time (Beekman *et al.* 2002). Bewick's swans are capable of flying 2000 km non-stop (Beekman *et al*. 2002), so they need at least one stopover to refuel and rest to complete their migration. The northern part of Denmark, the Baltic and the White Sea are known to be the major stopover sites during migration for Bewick's swans (Beekman *et al*. 2002).

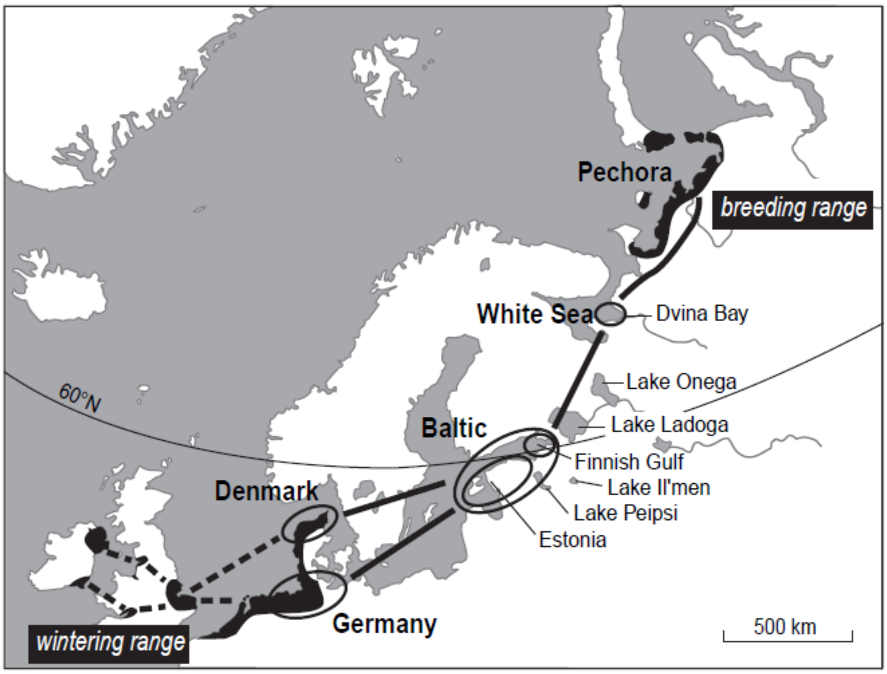

Figure 1. The winter and summer distribution of the north-western flyway population of Bewick's swans (*C. c. bewickii*), with the major stopovers indicated (Beekman *et al.* 2002).

2.3 Swan catching

2.3.1 Catching areas

The catching areas were chosen on the basis of: (1) flock size, (2) field type (sugar beets), (3) number of days that the Bewick's swans were on the field (should be three or more), (4) distribution of Bewick's swans over the field and (5) location of the field compared to houses (should be 750 meters away from the firing direction). Beet fields were chosen because the canon-net (§2.3.2) is less distinguishable and the birds can be encouraged to forage near the nets when there are a lot of beets in front of the net. The Bewick's swans used in this research have been captured during the winters of 2005-2009 (Table 1). In total 166 birds have been captured during nine catching attempts on different locations, where of 148 Bewick's swans were included in this research (Table 1, Figure 2).

2.3.2 Cannon-netting

Cannon-netting is a common used method for catching large numbers of birds, including geese, swans and ducks. Cannon-netting works by mean of explosive driven projectiles, which pull a net over a pre-determined area. Birds present in that area are covered by the net before they can escape. The net used in this research covered an area of 15x40 meters. With cannon-netting a representative sample of birds might be captured (BirdLife International 2006).

The cannon net was set up the evening before each catch day. The cannons (seven) are partly dug in the ground, with the barrel precisely aimed for deployment of the nets. The net is fired when the number of wanted individuals is in the pre-determined area, regarding that no birds are in the so-called safety zone. The safety zone is 3-5 meters from the edge of the pre-determined area, and should be free of birds due to the risk of casualties. The birds were removed from under the net, and put down in jute sacks.

2.3.3 Post-capture handling

The post-capture handling is the same for all birds, until the birds are assigned to one of the treatment groups (§2.3.4 and §2.3.5).

To start, all the individuals were fitted with a yellow neck-collar (with a unique 4 digit identifier code) and a stainless steel leg ring from Vogeltrekstation Arnhem. Then basic biometric data were measured, namely total head size (to nearest mm) and body mass (to nearest 0,1 kg). The birds were aged based on the plumage coloration and sexed based on cloacal examination. Due to the difficulties of cloacal sexing in the field, the birds were also sexed by the means of molecular sexing (except for the birds captured in 2005). Molecular sexing is commonly used to determine sex in sexually monomorphic birds.

Swabs were taken from the trachea and cloaca of each bird to test for current infection with a LPAI virus. Analysis of these swabs was conducted at Erasmus Medical Center, Rotterdam. There are four research groups discriminated and after the measurements and testing all birds were placed in one of these four research groups (§2.3.4 and §2.3.5, Table 2). Individuals found positive for LPAI virus were assigned to the naturally infected research group (Table 2). The swans which were not infected with a LPAI virus and not assigned to the experimental or the PBS treatment, function as the natural control swans (Table 2). The status of the birds (infected/uninfected) was unknown to the observers during the field period, to ensure objectivity.

Table 1. Overview of catching data, locations and numbers of Bewick's swans captured during the winters of 2005-2009.

	Date	Area	Location	Number of swans captured
1	18-12-2005	Wieringermeer	52º 48'30.96" N, 5º 5'51.00" E	12
2	10-12-2006	Wieringermeer	52º 47'30.84" N, 5º 4'59.16" E	12
3	16-12-2006	Wieringermeer	52º 52'15.96" N, 4º 59'24.00" E	16
4	16-12-2007	Wieringermeer	52º 52'59.88" N, 4º 58'0.12" E	22
5	17-12-2008	Flevopolder	52º 29'11.04" N, 5º 48'59.92" E	21
6	28-12-2008	Texel	53º 3'51.02" N, 4º 49'43.50" E	24
7	01-12-2009	Flevopolder	52º 30'36.00" N, 5º 49'14.52" E	15
8	11-12-2009	Lith	51º 47'23.28" N, 5º 26'10.68" E	12
9	30-12-2009	Wieringermeer	52º 49'42.60" N, 5º 5'49.92" E	14
Total number of Bewick's swans captured				148

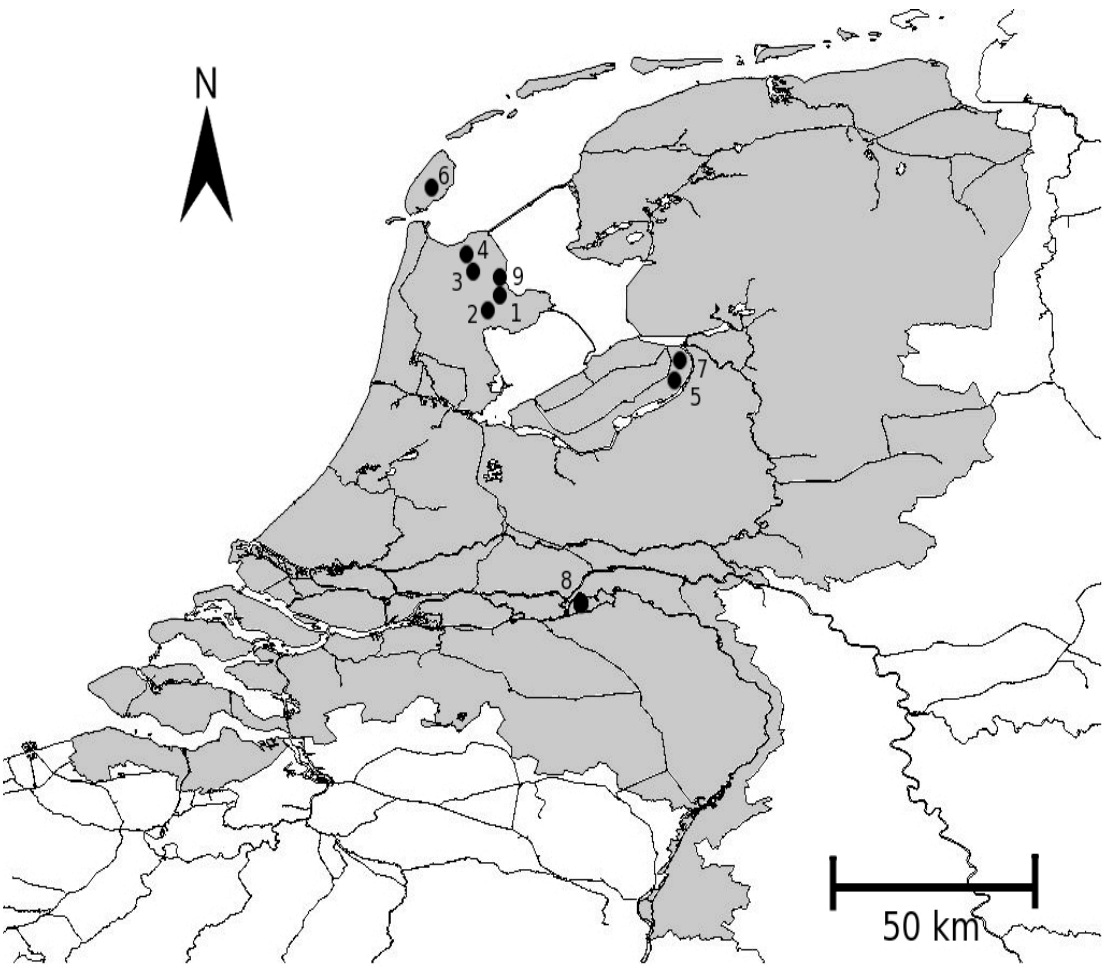

Figure 2. Locations of catching together with the date of capture. 1= Wieringermeer, 18-12-2005; 2= Wieringermeer,10-12-2006; 3= Wieringermeer, 16-12-2006; 4= Wieringermeer, 16-12-2007; 5= Flevopolder, 17-12-2008; 6= Texel, 28-12-2008; 7= Flevopolder, 01-12-2009; 8= Lith, 11-12-2009; 9= Wieringermeer, 30-12-2009.

2.3.4 Experimental infection H6N4

The processing of the Bewick's swans captured in the winters of 2008/2009 and 2009/2010, involved inoculating a number of Bewick's swans with a LPAI virus (H6N4) in Phosphate Buffered Saline (PBS, §2.3.5) solution. The virus was produced and stored by the Erasmus Medical Center in Rotterdam. The virus was transported on dry ice to the catching site on the day of capture. The inoculation was done with limited personnel present, in this case the lead scientist, an Erasmus Medical Centre employee and another scientist of the NIOO. The Bewick’s swans that were inoculated with the LPAI virus were assigned to the experimentally infected research group (Table 2).

Table 2. Overview of all Bewick’s swans included in this research, presented as the number of Bewick’s swans per research group (means and standard deviation).

		Experimentally infected	Naturally infected	Control	PBS	Total
Number of Swans		13	21	98	16	148
Mean body mass (in g)		5919,62 ±568,33	5795,48 ±790,56	6071,53 ±808,63	5782,69 ±680,42	5987,79 ±777,86
Mean skull length (in mm)		157,92 ±4,65	159,19 ±6,39	161,89 ±6,21	158,69 ±6,17	160,81 ±6,25
Mean condition index (in g)		129,59 ±573,78	-90,61 ±601,41	-19,00 ±651,93	-65,30 ±496,45	-21,11 ±620,16
Age	Adult	12 (92%)	9 (43%)	64 (65%)	16 (100%)	101 (68%)
	Yearling	1 (8%)	1 (5%)	8 (8%)		10 (7%)
	Juvenile		11 (52%)	26 (27%)		37 (25%)
Sex	Male	3 (23%)	14 (67%)	45 (46%)	6 (38%)	68 (46%)
	Female	10 (77%)	6 (29%)	51 (52%)	10 (62%)	77 (52%)
	Unknown		1 (4%)	2 (2%)		3 (2%)
Social status	Single	7 (54%)	2 (10%)	14 (14%)	9 (36%)	32 (22%)
	Paired	4 (31%)	1 (5%)	6 (6%)	5 (31%)	16 (11%)
	Family	2 (15%)	10 (48%)	32 (33%)	2 (13%)	46 (31%)
	Unknown		8 (37%)	46 (47%)		54 (36%)
Year of capture	2005		2 (9%)	10 (10%)		12 (8%)
	2006		5 (24%)	23 (24%)		28 (20%)
	2007		1 (5%)	21 (21%)		22 (15%)
	2008	5 (39%)	13 (62%)	19 (19%)	7 (47%)	45 (30%)
	2009	8 (61%)		25 (26%)	8 (53%)	41 (27%)

2.3.5 Inoculation with PBS

Phosphate Buffered Saline (PBS) is a water-based salt solution and commonly used in biological research. In the winters of 2008/2009 and 2009/2010 were in total 17 birds captured and assigned to this research group (Table 2). These birds have been treated exactly the same way as the experimentally infected individuals (§2.3.4), only they have been inoculated with a harmless PBS solution rather than a LPAI virus.

2.4 Resightings

One of the reasons why Bewick’s swans were chosen for this research is that they are relatively easy resighted due to their size and conspicuousness. The neck-collars, with the individual codes engraved on it, are readable up to 600 meters with the use of a telescope (20x60). Through a network of (mostly) amateur bird watchers, who entered their resightings on a special website (http://ncfs.nioo.knaw.nl/), the locations of the individual birds could be followed. The database of resightings contains data of birds captured in the winters 2005/2006 till 2009/2010. This database has been used to look at the dispersal of the individual birds.

2.5 GPS-loggers

In the winter of 2008/2009 and 2009/2010, 47 Bewick's swans were fitted with a GPS-logger, which was built into the neck-collar (Figure 2). A part of the data collection took place with the use of these GPS-loggers, which measures the position of the individual Bewick's swans. The GPS-loggers were built by Madebytheo (Nijmegen, The Netherlands). A GPS-logger consists of a miniature GPS-receiver and antenna, a Bluetooth transceiver and antenna, a flash storage device and a time-scheduled microprocessor, which controls data collection and transmission. A GPS-logger weights approximately 75 g. The data were collected at pre-scheduled times during the day and saved on the flash storage device of the GPS-logger. This occurred four times a day during migration of the swans and twice a day when not migrating. The data collected were: longitude, latitude, altitude, speed over ground, direction and ambient temperature. The accuracy of the spatial data is ± 50 metres. When a bird was resighted (§2.4), researchers went to that location to find the bird so that the data could be downloaded by using a computer with Bluetooth. Downloads took place when the individual bird was within range of the Bluetooth device (<300 meters). In total there are GPS data of 20 individual birds downloaded, of which 9 in the winters of 2008/2009 and 2009/2010 and 13 in the previous years. These data were used to look at the dispersal of the birds after being captured.

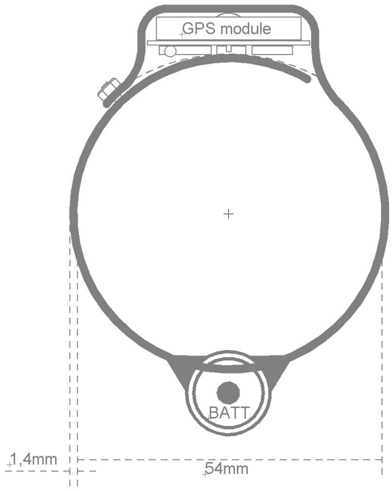

Figure 2. Cross section of a neck-collar with GPS-module built in (van Gils).

2.6 Abdominal Profile Index

An Abdominal Profile Index (API) is commonly used in waterfowl ecology to estimate the abdominal fat storage of a bird in the field (e.g. Owen 1981, Bowler 1994, Madsen & Klaassen 2006, Klaassen *et al.* 2005). Bowler (1994) showed that the API is positively correlated with the body mass of Bewick's swans, and thus a good predictor for body mass. This method is validated by comparing biometric data recorded upon catching, with the API of the same individual bird scored during field observations (Bowler 1994). The change in API over time was used to estimate a birds fuelling rate.

An API reflects the shape of the belly between the tail and legs. The API's were scaled between 1-6 (Bowler 1994, Figure 3), and estimated in the field with steps of ¼ unit.

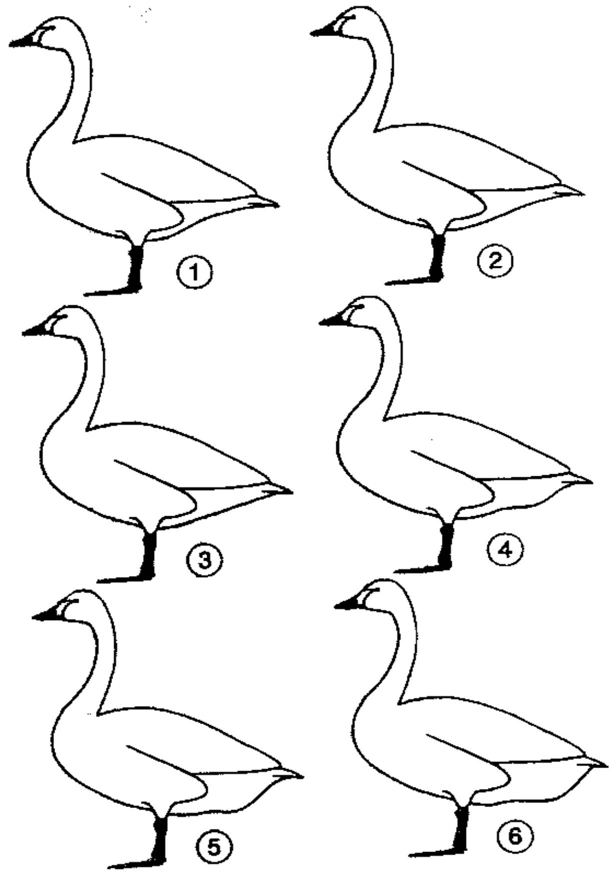

Figure 3. Classification (1-6) of abdominal profiles in Bewick's Swans used for field observations. 1= very concave; 2= concave; 3= straight; 4= convex; 5= very convex; 6= sagging (from Bowler 1994).

2.7 Data preparation

All collected data (resightings, GPS-downloads, API scores, biometric data and catching data) were put in one database to ensure data uniformity and ease of retrieval. First the data were combined from the different sources in Microsoft Excel and than imported into the database (PostgreSQL 8.4.2 with the spatial extension PostGIS 1.5; see appendix III for a flowchart). The data necessary for the analyses were extracted from the database and imported into PSAW Statistics 17.

2.7.1 Condition index

The main aim of using a condition index is to separate the aspects of body mass of a bird that are due to the structural body size, from the aspects that reflects fat and other energy reserves (Green 2001). The condition index is a common used method in many animal ecological studies (e.g. Pärt 1990, Lindén et al. 1992, Pietiäinen and Kolunen 1993, Veiga 1993). The condition indices are the residuals of the measured body mass (g) at capture minus the expected body mass (g). The expected body mass was calculated by performing a linear regression analysis using body mass (g) as dependent variable and a structural body size (in this case skull length in mm) as independent variable (Figure 4, R^2=0,364). The condition index (g) was used as a covariate in the analyses (§2.8).

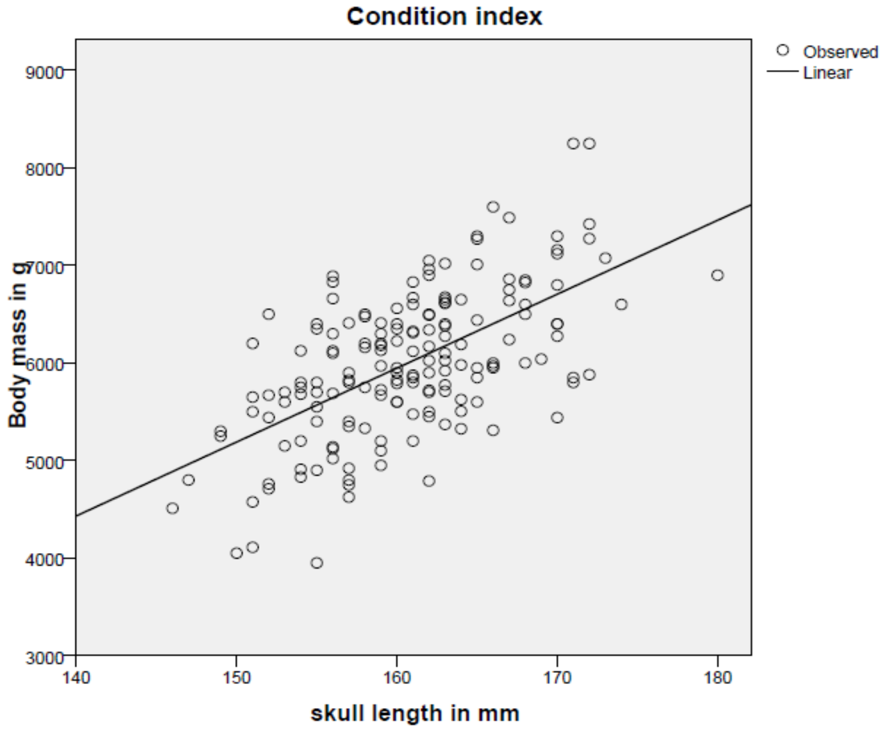

Figure 4. The condition index (g) was calculated by performing a linear regression analysis (R^2=0,364) using body mass in g (dependent variable) and skull length in mm (independent variable) (n=166). The regression slope represents the expected body mass (g) and the data points are the measured body mass (g) at capture.

2.7.2 Factors

Due to expected influence of different factors (social status, age, sex and year of capture) on the dispersal and the fuelling rate, they were included in the analyses.
The *social status* of the Bewick's swans was established in the field by observations. The birds were split up in three groups: single, paired or family. If the social status was not established in the field the swan was categorised as 'unknown'. A swan was single if no social interactions with other swans were observed. A swan was paired when social interactions with another swan were observed. A swan was categorised as family if it was accompanied with juveniles (regardless if the swan was paired or not).
The *age* of the Bewick's Swans was based on the plumage coloration. There were three groups discriminated, namely adults, yearlings and juveniles. Yearlings can be distinguished by some grey feathers on the crown. Juveniles have a grey plumage (Svensson *et al*. 2009).
The *sex* of the Bewick's Swan was determined by molecular sexing. So swans were categorized as males and females. The swans captured in the winter of 2005/2006 were not sexed by the means of molecular sexing. Therefore in some cases the sex remains unknown.
The Bewick's swans were also categorized by the *year of capture*, due to expected influences of the different seasons. So the swans were split up between 2005, 2006, 2007, 2008 and 2009.

2.8 Data analyses

As mentioned before, for the analyses of the two different parts of this research (winter movement and fuelling rate) the data of 148 Bewick's swans were analyzed. The data were based on their resightings, API-scores and on GPS downloads of 22 birds. There are four different research groups: (1) experimentally infected, (2) naturally infected, (3) control (uninfected) and (4) PBS (uninfected).

2.8.1 Analyses of variance with covariates (ANCOVA)

For both parts of this research a design with covariates is used (ANCOVA). This is because the winter movement and fuelling rate of the Bewick's swans are expected to be influenced by a number of variables (age, condition index, year of capture, social status and sex (§2.7.2)). To reduce the error of these variables and see how much of the variance can be explained by the different treatment groups, an ANCOVA was used for analyses in PASW statistics 17 (GLM, Univariate Analysis).

In the ANCOVA the main effects, as well as the two-way interactions are included. The least-significant interaction has been progressively removed from the model, to end up with a model were all the main effects as well as the significant interactions were included. How strong the influence of the interaction and main effects were is expressed with η_p^2 (partial eta squared). When η_p^2 has a value between 0,10 and 0,20 the effect was considered to be moderate. When $\eta_p^2 > 0,20$ the effect was considered to be strong. The η_p^2 also showed how much of the variance can be explained by that significant interaction.

When using ANCOVA, two assumptions about the data were made: (1) the collected data (dependent variable) are a random sample from a normal population; (2) in the population, all cell variances are the same. To test these assumptions a homogeneity test (Levene's test for Equality of Variances) is used and the residuals of the dependent variable are plotted to check for normal distribution.

2.8.2 Winter movement

The dispersal of an individual Bewick's swan is set out against number of days since capture. So day 0 is the day of capture, and day 30 is the 30^{th} day after being captured, regardless of the date or year the bird is captured. The dispersal is calculated as the minimum linear distance between the resighting location (or GPS location point) and the capture site. To visualize the trend of the dispersal per treatment group, a graph was made using a Loess fit line. A Loess line is a fit line using the iterative weighted least square of a proportion of the data points, in this research 75%. The function of the Loess line was uniform; giving each point the same weight. The mean dispersal per treatment group was calculated for 2, 5, 10, 14 and 30 days after capture. A logarithmic transformation of the dispersal has been used in the analysis, to improve the homogeneity. An Univariate Analysis (GLM) was used for analysis of the mean dispersal for the days 2, 5, 10, 14 and 30, were all main effects and all two-way interactions of all variables were taken into account.

2.8.3 Fuelling rate

The analysis of the fuelling rate of Bewick's swans has been based on the change in the visually scored API-units per day. This was calculated as the last API-score minus the first API-score divided by the number of days between these scores. An Univariate Analysis (GLM) has been used to test if there were any differences in the fuelling rate between the different research groups, when accounted for other factors (§ 2.7.2).

3. Results

The results are presented as mean and standard deviations, unless otherwise stated. In the first paragraph the results of the condition index are presented. For all birds captured (n=148) during five winters it is tested if their condition indices are significantly different between the research groups. In the second paragraph the results of the winter movement analysis are presented. The dispersal of the Bewick's swans is expressed as a natural logarithm of km. The last paragraph presents the results from the fuelling rate analysis. Because of the large difference in sample size, it is tested if the condition index was different for the four research groups, including only the swans which are in the fuelling rate analysis.

3.1 Condition Index

The different treatments of the Bewick's swans showed no effect on their condition indices (Table 3). As expected, the different age classes of the Bewick's swans had a strong effect on their condition indices (Table 3), and it explained 29% of the variance. Not surprisingly adult swans (193,27 g ± 584,06 n=101) had a higher condition index than yearlings (-197,97 g ±482,35 n=10) and juveniles (-558,50 g ±360,94 n=37). The difference however was only significant between adult swans and juveniles (Bonferroni, p<0,001), but not between adults and yearlings (p=0,105) or yearlings and juveniles (p=0,075).

Females (-157,40 g ±607,82 n=77) had a significant lower condition index at capture than the males (144,89 g ±608,27 n=68) (Bonferroni, p=0,01). The effect however is considered to be weak and it only explains 6% of the variance in the condition indices.

Table 3. GLM test results of the effect of treatment group on the condition index (g) of Bewick's swans (n=148). Other main effects are also included.

	df	F	p-value	η_p^2
Treatment group	3, 133	1,068	0,365	0,024
Age	2, 133	27,466	<0,001	0,292
Sex	2, 133	4,554	0,012	0,064
Social status	3, 133	0,783	0,505	0,017
Capture year	4, 133	0,498	0,737	0,015

3.1 Winter movement

The data of the winter movement of Bewick's swans were obtained by combining logged GPS-positions (449 data points, n=22) and resightings (861 data points, n=141). The combined data consists of 1191 data points (n=143). Some of the data of the resightings were not included as there already was a logged GPS-download position for that day. To check if the data could be combined, two box plots were made to present if there were differences in the logarithmic dispersal per day over the first 30 days after capture, for the two data sets (Figure 5).

There were no major differences in the logarithmic dispersal observed between resightings and logged GPS-positions, so both data sets were put together (Figure 6). Remarkably, resightings showed more outliers than logged GPS-positions. This indicates that there is more variation and extreme values in the data based on resightings than in data based on logged GPS-positions of Bewick's swans.

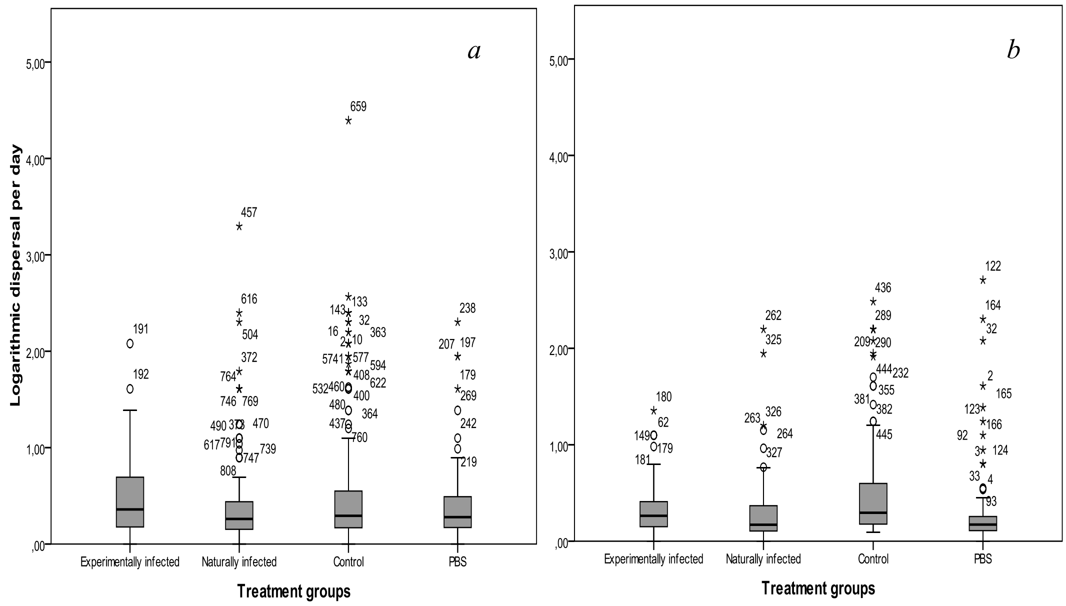

Figure 5. Box plots of the logarithmic dispersal per day over the period of 30 days after capture for resightings (a; experimentally infected median=0,358, naturally infected median=0,260, control median=0,293 and PBS median=0,279) and GPS-downloads (b; experimentally infected median=0,264, naturally infected median=0,171, control median=0,295 and PBS median=0,172).

Trend analysis

Surprisingly, Bewick's swans on average tended to stay within the capture area for the first nine days (Figure 6), though large individual differences started to show from day five. Day nine showed a slight change in the dispersal trend between the treatment groups. Bewick's swans in the control group (n=94) started to forage further away from the catching area and kept doing so until the end of the period. The experimentally infected Bewick's swans (n=13) were seen further away from the catching area around day twelve and followed the swans in the control group. The Bewick's swans that were in the naturally infected group (n=21) and in the PBS group (n=15) stayed closer to the catching area, however the swans of the PBS group tended to move slightly further away from the catching area around day twelve.

Because naturally infected swans were already infected at the moment of capture, it was of interest to see if their infection affected their dispersal right after being captured, so day two is examined. For the experimentally infected swans (which were infected right after being captured) it was expected to see the effects of their infection in the first week after capture, so day five and ten were checked to see if there was an effect of the treatment of swans with Avian Influenza. To test for an intermediate to long-term effect of the different treatments, days 14 and 30 were examined. For all analyses of winter movement the natural logarithm of the dispersal was used, to enhance the normal distribution and homogeneity of the dispersal data.

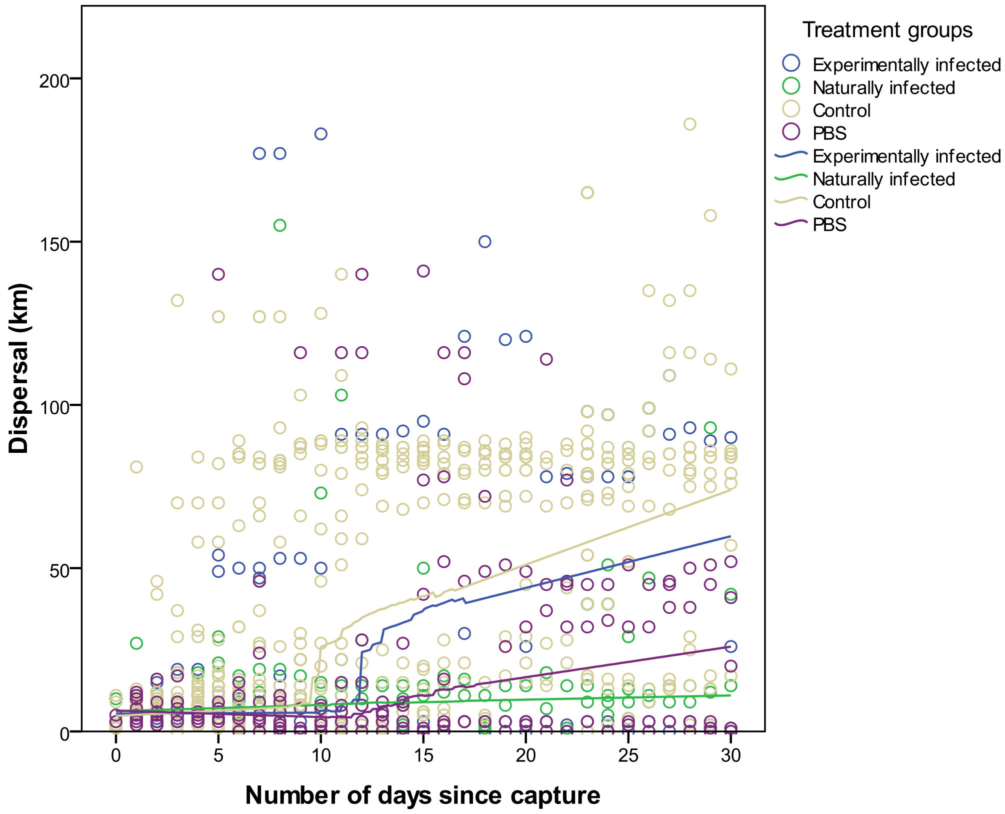

Figure 6. The mean dispersal of Bewick's Swans plotted against days after capture based on the GPS-downloads as well as resightings (Loes uniform 0,75). The dispersal is presented per research group based on resighting and GPS-downloads (experimentally infected n=13, naturally infected n=21, control n=94 and PBS n=15).

In the first 30 days after capture (and infection) there was no significant difference in dispersal found between the four treatment groups (experimentally infected, naturally infected, control and PBS). However in some cases (day 10 and 14) a strong effect of the treatment groups on the dispersal was suggested, but no significant differences showed. This could mean that the sample size in these cases have been too small. Remarkably, the experimentally infected swans decreased in mean dispersal after day 14 (Table 4). The median of the dispersal showed that swans in the control group dispersed away from the catching area at day 10 and that swans in the naturally infected and control group dispersed from the catching site at day 30, while the experimentally infected Bewick's swans stayed the entire 30 days in the catching area. The dispersal per day is further explained below.

Table 4. The mean and median for the experimental groups per days after capture.

	Experimentally infected		Naturally infected		Control		PBS	
	Mean	Median	Mean	Median	Mean	Median	Mean	Median
Day 2	6,38	5,0	8,00	7,5	8,59	6,0	6,29	5,0
Day 5	28,78	7,0	10,67	8,0	28,04	12,0	24,71	6,0
Day 10	60,00	7,0	13,60	7,0	39,40	20,0	4,00	3,0
Day 14	33,67	9,0	14,43	8,0	42,23	21,0	8,50	5,5
Day 30	29,25	13,5	28,00	28,0	54,86	77,5	22,80	20,0

Dispersal on day 2

Two days after capture the Bewick's swans in the control group grazed further away (8,59 km ±8,834 n=39), closely followed by the naturally infected swans (8,00 km ±3,232 n=10). The experimentally infected and PBS swans stayed closer to the catching area than the swans in the control group and naturally infected swans, both grazed at the same distance (respectively 6,43 km ±5,062 n=7 and 6,29 km ±4,889 n=7). There were no differences in dispersal between the treatment groups (Table 5), but all two-way interactions with the treatment groups and another factor (year of capture, age, social status, sex and condition index). Although there are quite some two-way interactions most birds are still in the catching area.

Table 5. GLM test results of the effects of the treatment groups on the dispersal at day 2 of Bewick's swans (n=63). Other main effects and significant two-way interactions were included.

	df	F	*p*-value	η_p^2
Treatment group	3, 9,751	0,416	0,745	0,114
Year of capture	4, 2,706	0,315	0,852	0,318
Age	2, 2,031	1,148	0,464	0,531
Social status	3, 5,225	0,517	0,688	0,229
Sex	2, 5,647	0,892	0,461	0,240
Condition index	1, 24	11,245	0,003	0,319
Treatment group x age	2, 24	6,224	0,007	0,342
Social status x condition index	3, 24	6,785	0,002	0,459
Treatment group x condition index	3, 24	5,754	0,004	0,418
Social status x sex	3, 24	6,488	0,002	0,448
Treatment group x sex	3, 24	10,746	<0,001	0,573
Treatment group x social status	4, 24	13,534	<0,001	0,693
Treatment group x year	3, 24	8,309	0,001	0,509

Dispersal on day 5

The dispersal at day five for the experimentally infected was 20,00 km (±21,67 n=7), for naturally infected 10,67 km (±7,24 n=12), for the control group 28,04 km (±38,15 n=23) and for swans in the PBS group 24,71 km (±50,88 n=7). A possible moderate effect was suggested (η_p^2=0,180; Table 6), because the sample size might have been too small there was no significant (*p*=0,07) difference in dispersal between the treatment groups. This indicates that swans in the experimentally infected, control and PBS groups on average are dispersed outside the catching area, while swans in the naturally infected group were still within the catching area.

Table 6. GLM test results of the effect of treatment groups on the dispersal at day 5 of Bewick's swans (n=49). Other main effects are included.

	df	F	*p*-value	η_p^2
Treatment group	3, 35	2,562	0,070	0,180
Year of capture	2, 35	1,027	0,368	0,055
Age	2, 35	0,344	0,711	0,019
Social status	2, 35	0,368	0,695	0,021
Sex	2, 35	0,032	0,969	0,002
Condition index	1, 35	0,039	0,845	0,001

Dispersal on day 10

Ten days after capture the control individuals dispersed 39,40 km (±40,54 n=20) from the catching area. The experimentally infected individuals grazed even further away (60,00 km ±84,93 n=4). The naturally infected swans stayed on 13,60 km (±21,41 n=10) from the catching area. The Bewick's swans in the PBS group dispersed 4,00 km (±3,391 n=5). At day ten after capture the experimentally infected and control Bewick's swans were resighted further away than on day 5, surprisingly the swans in the PBS group were only resighted within the catching area as were the naturally infected swans. A strong effect was suggested (η_p^2=0,202) of the treatment groups on the dispersal (Table 7), but the sample size might have been too small for a significant difference.

Table 7. GLM test results of the effect of treatment group on the dispersal at day 8 of Bewick's swans (n=39). Other main effects are included.

	df	F	*p*-value	η_p^2
Treatment group	2, 22	1,855	0,167	0,202
Year of capture	3, 22	2,190	0,118	0,230
Age	2, 22	0,278	0,760	0,025
Social status	2, 22	0,462	0,636	0,040
Sex	2, 22	0,038	0,963	0,003
Condition index	1, 22	0,681	0,418	0,030

Dispersal on day 14

At day 14 after capture of the Bewick's swans, individuals in the control group dispersed 42,23 km (±38,79 n=13) from the catching area. Surprisingly, the experimentally infected Bewick's swans were seen at 33,67 km (±50,72 n=3) from the catching area while at day ten they were at 60 km. Naturally infected swans stayed the closest to the catching area with a mean dispersal of 8,33 km (±3,88 n=6). Swans that were in the PBS group had a dispersal of 8,50 km (±9,586 n=6). At day 14 an effect of the year of capture is seen (*p*=0,02), this indicates that in different winters the dispersal differed. The dispersal between the treatment groups suggested a strong effect (η_p^2=0,438), which could indicate that the sample size was too small (Table 8).

Table 8. GLM test results of the effect of treatment group on the dispersal at day 14 of Bewick's swans (n=27). Other main effects are also included.

	df	F	p-value	η_p^2
Treatment group	3, 11	2,863	0,085	0,438
Year of capture	4, 11	4,580	0,020	0,625
Age	2, 11	0,482	0,630	0,081
Social status	3, 11	2,048	0,166	0,358
Sex	2, 11	1,469	0,272	0,211
Condition index	1, 11	0,375	0,553	0,033

Dispersal on day 30

It was surprising to see that swans in the experimentally infected group were seen closer to the catching site on day 30 than on day 14 (29,25 km ±42,25 n=4). The swans in the other research groups did move away from the catching area (naturally infected: 28,00 ±19,80 n=2, control: 54,86 km ±40,86 n=14 and PBS: 22,80 km ±23,38 n=5).

3.3 Fuelling rate

3.3.1 Condition index

The different treatments of the Bewick's swans showed a strong effect on their condition indices of the Swans included for the fuelling rate analysis (Table 9, Appendix III), and 21% of the variance in condition indices could be explained by this effect. It turned out that the experimentally infected swans had a significant higher condition index than the naturally infected swans (Bonferroni, p=0,04) and the control individuals (p=0,07). The swans did not significantly differ in condition indices between the other treatment groups (p>0,05).

Table 9. GLM test results of the effect of treatment group on the condition index (g) of Bewick's swans (n=60). Other main effects are also included.

	df	F	p-value	η_p^2
Treatment group	3, 47	4,215	0,010	0,212
Age	2, 47	20,870	<0,001	0,470
Sex	2, 47	2,459	0,096	0,095
Social status	2, 47	2,171	0,125	0,085
Capture year	2, 47	5,270	0,009	0,183

The different age classes of the Bewick's swans had a strong effect on their condition indices (Table 9), and it explained 47% of the variance. Not surprisingly adult swans (127,69 g ± 393,68 n=42) had a higher condition index than yearlings (-200,67 g ±329,55 n=3) and juveniles (-529,81 g ±261,92 n=15). The difference however was only significant between adult swans and juveniles (Bonferroni, p<0,001), but not between adults and yearlings (p=0,148) or yearlings and juveniles (p=1,0).

The fluctuations in weather conditions during the five different winters, did show a moderate effect on the condition indices of the Swans. The differences in years explained 17% of the variance in condition index (Table 9). The swans captured in 2008 had a significant different condition index than birds captured in 2009 (Bonferroni, p=0,046). The swans captured in the other years didn't show significant differences (p>0,05).

3.3.2 Change in API-units per day

The fuelling rate is expressed as the change in the visually scored API unites per day (Figure 3, §2.6). Surprisingly the swans which were experimentally infected increased the most in API with 0,029 unit per day (±0,043, n=10). The control swans increased in API with 0,008 unit per day (±0,044, n=31), the PBS swans with 0,018 unit per day (± 0,055, n=12) and the naturally infected swans with 0,001 unit per day (±0,009, n=7) (Figure 8).
The period between the first and the last API-score was for the control group 34,32 days (±27,62), for the PBS group 36,17 days (±15,51), for the experimentally infected 33,30 days (± 23,97) and for the naturally infected group 52,57 days (±10,71).
There were no significant differences in the fuelling rates of Bewick's swans when controlled for the condition index and other factors between the four different research groups (Table 10).

Table 10. GLM test results of the effect of treatment group on the fuelling rate (expressed as the change in visually scored API's) in Bewick's swans (n=63). Other main effects are included.

	df	F	p-value	η_p^2
Treatment	3, 46	0,096	0,962	0,006
Capture year	2, 46	2,222	0,120	0,088
Social status	2, 46	0,453	0,639	0,019
Sex	2, 46	0,164	0,850	0,007
Age	2, 46	0,428	0,654	0,018
Condition Index	1, 46	0,071	0,792	0,002

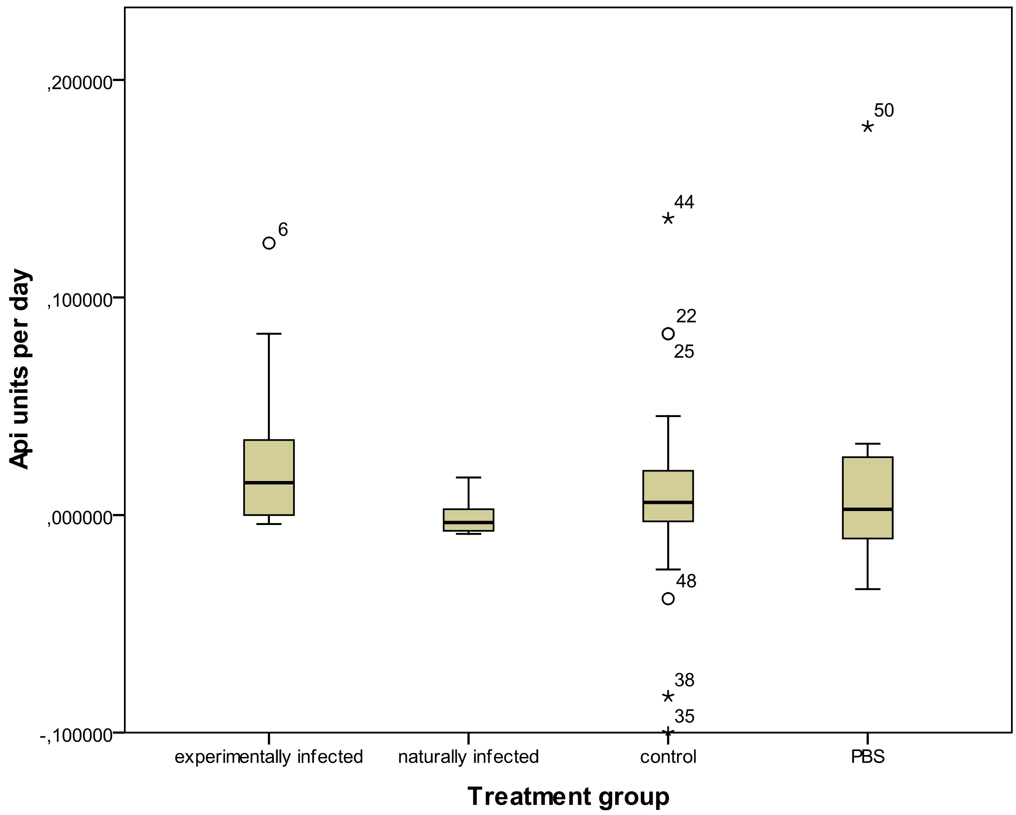

Figure 8. The median fuelling rate (experimentally infected is 0,015 n=10, naturally infected is -0,003 n=7, control is 0,006 n=31 and PBS is 0,003 n=12), expressed as the change in the visually scored API-units per day in Bewick's swans (n=60).

4. Discussion

4.1 Condition index

Given the fact that the experimentally infected individuals were inoculated with the virus after capture (and thus not suffering from a virus infection at the moment of capture), it was expected that there was no difference in condition index at capture between the experimentally infected, PBS and the control individuals (the swans not found naturally infected at the time of capture). Naturally infected swans were expected to have a lower condition index, due to the virus they were struggling with.

In our study, there were no significant differences in the condition indices of Bewick's swans between the different research groups. Van Gils *et al.* (2007) showed no difference in body mass between the naturally infected and the uninfected Bewick's swans (body mass was controlled for body size and age). Latorre-Margalev *et al.* (2009) studied the effect of LPAI infection in wild Mallards (*Anas platyrhynchos*), and showed a significant difference in body mass between naturally infected and uninfected Mallards. Van Gils *et al.* (2007) suggested that the two naturally infected Bewick's swans included in his research, are presumably infected with a LPAI-virus infection shortly before capture, which could explain why there was no significant difference in body mass between infected and uninfected swans. For the Bewick's swans used in our research it remains unknown exactly when the Bewick's swans actually got infected with the virus, but it could be possible that they were infected shortly (maybe a couple of days) before capture, which could explain that there were no differences between the four research groups in condition index.

The condition index is a commonly used method to correct for the body mass which is due to structural body size and what part of the body mass is due to other energy stores or fat (Green *et al.* 2001). Our results showed a strong effect of age on the condition index. Green *et al.* (2001) mentioned that it is favourable to calculate a condition index for the different age classes as well as for both sexes. Because we wanted to see how much of the variance in condition index could be explained by other factors, such as the age, sex, social status and year of capture of the Bewick's swan, in comparison with how much of the variance could be explained by the difference in treatment, we calculated the condition index for each swan regardless to sex or age. If we choose to do not so, it wouldn't be possible to see how much of the variance could be accounted for by these factors.

4.2 Winter movement

During the first 30 days after capture (and infection) there was no significant difference in dispersal between the four research groups (experimentally infected, naturally infected, PBS and control) of Bewick's swans. However in some cases (days 10 and 14) a strong effect of the treatment groups on the dispersal was suggested but no significant differences showed. The median of dispersal (rather than the mean dispersal) showed that the experimentally infected individuals dispersed less far than the control individuals. Reinmann & Filzmoser (2000) suggested that the median should be used when dealing with environmental data, as it is less influenced by extreme cases, and environmental data are almost never normal distributed. We indeed observed extreme cases when combining the data from GPS-downloads and resightings (Figure 5, §3.2). When looking at the median for both the experimentally infected and PBS swans, there is only a difference at day 30 when the PBS swans have left the catching site. As the prevalence of the avian influenza virus is unknown in Bewick's swans, it is uncertain to tell whether the experimentally infected swans stayed within the catching area due to the infection or due to environmental conditions. Latorre-Margalef *et al.* (2009) showed that the duration of shedding was 8,3 days in mallards, as this is highly species specific (Garamszegi & Møller 2007) it might only be used as an indication of the shedding time. Further research should reveal the duration of shedding in Bewick's swans. If the shedding is indeed around the same period as in Mallards as it is in Bewick's swans, the dispersal is not affected by an Avian influenza infection. Kleijn *et al.* (2010) found that distance travelled was not influenced by an Avian Influenza infection in greater white-fronted geese, also supporting this conclusion.

The suggested effects of social status and year of capture increases in time, these factors, beside the infection, might in time be more influencing the dispersal of Bewick's swans than the infection. It is known of Bewick's Swans that 24% of their movements is socially related (Klaassen *et al.* 2006). The social status also influences the length of the movement of individual Swans. Within Bewick's Swans there is a social hierarchy, were singles are more or less scouts, and paired Bewick's Swans and families follow the single birds. This also implies that single birds have a higher dispersal than swans that are paired or form a family. This does imply that the suggested effect found is influencing the dispersal of Bewick's swans.
The prevalence of an Avian influenza infection varies from year to year (Olsen *et al.* 2006, Garamszegi & Møller 2007). The environmental conditions also vary between winters, which can also influence the dispersal of Bewick's swans. White-fronted geese dispersed southwards in severe winters (Meire & Kuiken 1991), indicating that the dispersal is influenced by the severity of the winter. The severity of the winter and difference in prevalence of Avian influenza, support a possible effect on the dispersal of the year of capture.

The dispersal of Bewick's swans didn't show an effect of the Avian influenza infection. This suggests a relative short shedding time or mild effects of the Avian influenza infection, which is in line with previous found conclusions (Latorre-Margalev *et al.* 2009, Olsen 2006, Garamszegi & Møller 2007, Kleijn *et al.* 2010), but not with van Gils *et al.* (2007) who found that naturally infected Bewick's swans had reduced fuelling and feeding rates and a delayed departure of spring migration.

4.3 Fuelling rate

Because of the different sample sizes used for the analysis of winter movements and fuelling rates, we have also tested if the condition index was significantly different between the research groups for the Bewick's swans (n=60) which are included in the fuelling rate analysis.
Given the fact that the experimentally infected individuals were inoculated with the virus after capture (and thus not suffering from a virus infection at the moment of capture), it was expected that there was no difference in condition index at capture between the experimentally infected, PBS and the control individuals (the swans not found naturally infected at the time of capture).
Surprisingly, it turned out that the swans, which were experimentally infected, had a significantly higher condition index (282,76 g ± 335,07, n=10) than the naturally infected swans (-272,82 g ± 330,46, n=7) and the control individuals (-105,17 g ±

501,98, n=31). Because of the random assignment of the swans to the treatment groups (with exception of the naturally infected swans), this is most likely to be a coincidence. The low condition index at capture of swans suffering from a natural infection can mean two things: (1) either the bad condition is a consequence of the infection or (2) swans with a bad condition are easier infected with the virus. The fact that the experimentally infected swans had a significant higher condition index than the naturally infected swans and a suggested higher fuelling rate, could suggest that the virus is the consequence rather than the cause.

The fuelling rate of the Bewick's swans is expressed as the visually scored changes in API units per day. Van Gils *et al.* (2007) showed that there was a negative correlation of the fuelling rate in naturally infected Bewick's swans, but he had a small sample size of only two naturally infected Bewick's swans. When looking at the mean fuelling rate (ignoring the variances) the data suggests the same findings as van Gils *et al.* (2007), namely that naturally infected swans have a lower fuelling rate then uninfected Bewick's swans, however this difference was not significant. Surprisingly, the experimentally infected Bewick's swans had the highest fuelling rate of all swans.

As displayed in figure 8 (§3.3.4), there was a lot of variance in the fuelling rate data. It was expected that (some of) these variance could be explained by factors, such as age, sex, social status, year and/or the condition index. The analysis didn't show any significant effects of the main factors or two-way interactions. While in the analyses of Bowler (1994) the variance in the observed API-scores were significantly reduced (for both sexes) by year, time (in half-month intervals), social status and, for females only, the dominance rank (expressed as the outcome of aggressive interactions between swans).

Bowler (1994) also showed that the API-scores were significantly higher for females throughout the winter than for males. While in our research the factor sex had no effect on the change in API-units per day. Another remarkable fact is that cygnets (juveniles) are known to put on weight more rapidly in the winter than other age categories (Evans & Kear 1978), so an effect was expected of age on the fuelling rate as well, but no effect showed in our analysis.

As API scores were positively correlated with the change in body mass (Bowler 1994), it was expected that the experimentally infected should have had the highest API scores as well. Though this did not show in the mean API score or the median of the API scores. Bowler (1994) did mention that there was a considerably overlap between values of adjacent profiles, this indicates that predictions for single birds may not be very precise.

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Appendix I. Research group for winter movement analysis

Table 11. Number of Bewick's swans per research group for the analysis of winter movement presented with means and standard deviation.

		Experimentally infected	Naturally infected	Control	PBS	Total
Number of swans		13	21	94	15	143
Mean body mass (in g)		5919,62 ±568,33	5795,48 ±790,56	6065,37 ±820,41	5789,00 ±703,81	5983,50 ±786,77
Mean skull length (in mm)		157,92 ±4,65	159,19 ±6,39	161,87 ±6.25	158,87 ±6,35	160,80 ±6,28
Mean condition index (in g)		129,60 ±573,78	-90,61 ±601,41	-23,99 ±650,42	-72,54 ±512,99	-24,90 ±620,35
Age	Adult	12 (92%)	9 (43%)	61 (65%)	15 (100%)	97 (68%)
	Yearling	1 (8%)	1 (5%)	7 (7%)		9 (6%)
	Juvenile		11 (52%)	26 (28%)		37 (26%)
Sex	Male	3 (23%)	14 (67%)	44 (47%)	6 (40%)	67 (47%)
	Female	10 (77%)	6 (29%)	49 (52%)	9 (60%)	74 (52%)
	Unknown		1 (4%)	1 (1%)		2 (1%)
Social status	Single	7 (54%)	2 (10%)	12 (13%)	8 (53%)	29 (20%)
	Paired	4 (30%)	1 (5%)	5 (5%)	5 (33%)	15 (11%)
	Family	2 (16%)	10 (48%)	32 (34%)	2 (14%)	46 (32%)
	Unknown		8 (37%)	45 (48%)		53 (37%)
Year of capture	2005		2 (10%)	9 (10%)		11 (8%)
	2006		5 (24%)	23 (25%)		28 (19%)
	2007		1 (5%)	21 (22%)		22 (15%)
	2008	5 (39%)	13 (61%)	17 (18%)	7 (47%)	42 (29%)
	2009	8 (61%)		24 (25%)	8 (53%)	40 (28%)

Appendix II. Research group for fuelling rate analysis

Table 12. Number of Bewick's swans per research group for the analysis of fuelling rate presented with means and standard deviation.

		Experimentally infected	Naturally infected	Control	PBS	Total
Number of swans		10	7	31	12	60
Mean body mass (in g)		5895,00 ±631,37	5635,71 ±847,64	5914,52 ±869,65	5805,25 ±573,06	5856,88 ±766,81
Mean skull length (in mm)		157,30 ±5,03	157,14 ±7,03	160,94 ±6,54	160,25 ±5,01	159,75 ±6,16
Mean condition index (in g)		282,76 ±335,08	-272,82 ±358,93	-105,17 ±501,98	-70,31 ±381,39	-53,10 ±458,05
Age	Adult	9 (90%)	3 (43%)	18 (58%)	12 (100%)	42 (70%)
	Yearling	1 (10%)	1 (14%)	1 (3%)		3 (5%)
	Juvenile		3 (43%)	12 (39%)		15 (15%)
Sex	Male	3 (30%)	4 (57%)	12 (39%)	5 (42%)	24 (40%)
	Female	7 (10%)	2 (29%)	19 (61%)	7 (58%)	35 (58%)
	Unknown		1 (14%)			1 (2%)
Social status	Single	7 (70%)	2 (29%)	5 (16%)	7 (58%)	21 (35%)
	Paired	2 (20%)	3 (42%)	2 (7%)	4 (33%)	8 (13%)
	Family	1 (10%)	2 (29%)	18 (58%)	1 (9%)	23 (39%)
	Unknown			6 (19%)		8 (13%)
Year of capture	2005		2 (29%)	4 (13%)		6 (10%)
	2007			2 (7%)		2 (3%)
	2008	3 (30%)	5 (71%)	6 (20%)	7 (58%)	21 (35%)
	2009	7 (70%)		19 (60%)	5 (42%)	31 (52%)
Moment API-score (days after capture)	First	8,60 ±9,26	6,29 ±7,41	11,13 ±14,39	8,42 ±5,63	9,60 ±11,53
	Last	41,90 ±22,61	58,86 ±3,53	45,45 ±24,94	44,58 ±13,77	46,25 ±21,30
Change in API-units per day		0,029 ±0,043	-0,001 ±0,010	0,008 ±0,044	0,018 ±0,055	0,012 ±0,044
Days between measurements		33,30 ±22,97	52,57 ±10,71	34,32 ±27,62	36,17 ±15,51	36,65 ±23,66

Appendix III. Flowchart of input data in database.