INDIVIDUAL FITNESS CORRELATES IN CONSECUTIVE YEARS OF PAIR BOND IN UPLAND GEESE

DISSERTATION

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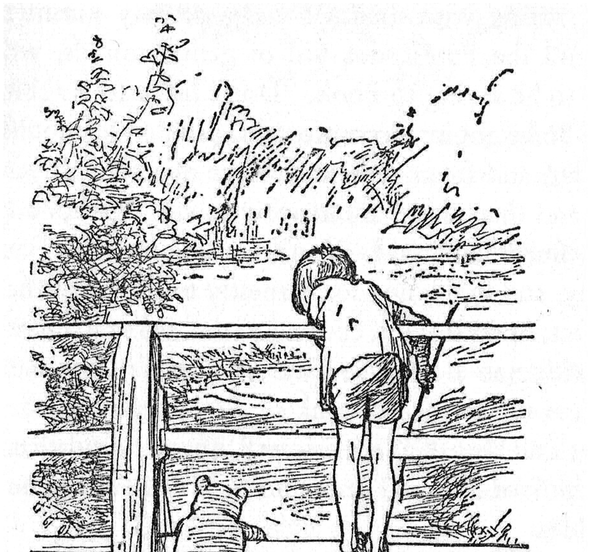

“Sometimes, if you stand on the bottom rail of a bridge and lean over to watch the river slipping slowly away beneath you, you will suddenly know everything there is to be known.“

Winnie the Pooh

CONTENTS

ONE

General introduction

GENERAL INTRODUCTION

To explain, why a certain species is what it is and lives where it does, it is necessary to understand the evolutionary processes that lead to its current appearance and distribution. In this thesis I describe a variety of individual characters and their relation to fitness in both males and females in a population of wild Upland geese (*Chloephaga picta leucoptera*) on the Falkland Islands.

Natural and sexual selection

Individuals will survive, reproduce and leave different numbers of descendants, depending upon how well they are adapted to current environmental conditions. In this sense, some individuals are fitter than others; they make a greater contribution to the gene pool of the next generation. Traits that increase the fitness of an individual are selected for, others that decrease fitness are selected against and traits that neither increase nor decrease fitness are selectively neutral (Fisher 1930). The change of heritable characteristics of a population during the course of time is called evolution by natural selection. This concept was first published by Darwin in 1859 and later refined by introducing sexual selection as a special process shaping mechanisms that function shortly before or at the time of mating and serve in the process of obtaining mates (Darwin 1871). Darwin regarded it basically different from most forms of natural selection in that it does not increase survival but reproductive success of individuals. Using Darwin's own words, sexual selection can be divided into two distinct processes, "the power to charm the females" (intersexual selection), and "the power to conquer other males in battle" (intrasexual selection). Today it is acknowledged that both natural and sexual selection are just different aspects of one process, because both survival and reproductive success influence the lifetime reproductive output and thereby fitness of individuals.

Phenotypic variation - the basis for selection

For both natural and sexual selection to work, inter-individual variation is fundamental. This can be caused by mutation, recombination and gene flow between populations. A specific trait in a population can evolve through selection only if the variations affect individual fitness and between-individual variation is consistent or highly repeatable over time.

Selection acts upon the phenotype of organisms, i.e. any observable characteristic or trait (morphology, development, biochemical or physiological properties, behaviour and products of behaviour). The phenotype of an individual results from the expression of its inherited genes and the influence of environmental and developmental factors. Phenotypic differences between individuals within a population that are correlated with fitness can indicate which selection pressures are currently working or have been working in the past. We thereby can try to explain which selective forces led to the current appearance and behaviour of individuals. Knowledge about fitness correlates can be used to manage populations or to predict the potential impact of changes in environmental conditions due to natural or anthropogenic causes.

Only heritable traits can evolve through selection

The determination of fitness correlates in field studies is only the first step to understand how selection may have worked in a particular species. After the description of phenotypic differences that are related to variations in fitness, it is necessary to investigate whether these traits could have been shaped by selection. As only selection acting on the genetic component of a trait will lead to an evolutionary response (Kruuk 2004), while selection on the nonheritable environmental component of the phenotype will not (Price et al. 1988; Rausher 1992), selection can only optimize traits that are heritable.

Heritability is defined as the proportion of total phenotypic variation due to all genetic effects. Detecting the heritability of traits in field studies is not always easy. One method we used in this study is to look at the repeatability of measured characters. Repeatability is the proportion of phenotypic variation that can be attributed to between-subject (or between-group) variation and an important index for quantifying the accuracy of measurements and the constancy of phenotypes (Lessells and Boag 1987). Whereas heritability indicates purely genetic differences, the repeatability index incorporates both genetic and environmental sources of variation. It is assumed to set the approximate upper limit for heritability (Falconer and Mackay 1996; Dohm 2002)

Traits that show high inter-individual variation and high repeatability levels could have potentially evolved through natural or sexual selection.

Why study fitness correlates in Upland geese?

Studies of selection in the wild have continually increased since Darwin (1859) introduced his theory 150 years ago. A first review of field studies was published by Endler in his influential book 'Natural selection in the wild' (1986). Two more recent reviews by Kingsolver (2001) and Siepielski et al. (2009) both found a very similar strength of selection on traits (averaged over years within a dataset) and give an up-to-date overview over selection studies. Despite the large number of studies that can be found for birds, there is a considerable bias towards studies in Passeriformes and species with a socially monogamous mating system with seasonally changing partners (Table 1). Also, most studies concentrate on morphology and life history, while studies focussing on selection on physiological traits are still missing (Table 2).

Table 1. Studies on selection in wild bird populations included in recent reviews.

	Kingsolver (2001)	Siepielski (2009)
Order		
Passeriformes	14	17
Anseriformes	0	1
Charadriiformes	0	2
Galliformes	0	1
Mating system		
Monogamous (seasonal)	12	14
Monogamous (long-term)	0	1
Polygynous	2	6

However, it is dangerous to build the empirical study of natural selection and microevolutionary biology primarily on data from certain species and traits. The breadth of applicability of generalizations and population genetic models should be tested across taxa. As far as possible we should determine whether or not different processes are important in organisms with a variety of life histories.

In the present study series I investigated a free-ranging population of Upland geese in the New Island Nature Reserve, Falkland Islands. Upland geese belong to the order Anseriformes, they form stable social monogamous pair bonds over several years and show a strong sexual dimorphism in both size and colouration, a combination underrepresented in the current literature. In this thesis I describe the relationship of several morphological and physiological traits to aspects of fitness in both males and females of this species.

Table 2. Summary of the selection database in Siepielski et al. (2009) – Table adapted

	Number of items in the database
Studies	89
Selection coefficients	5519
Linear differentials	1989
Linear gradients	1989
Quadratic differentials	776
Quadratic gradients	765
Species	73
Genera	61
Taxon type	
Invertebrates	482 (studies = 13)
Plants	365 (studies = 28)
Vertebrates	2567 (studies = 48)
Total types of selection	
Sexual selection	512
Natural selection	2902
Trait type	
Behavioural	21
Life history	1244
Morphological	1839
Principal components	310
Physiological	0

The study of individual fitness correlates and their repeatability under natural conditions ideally requires a study system in which individuals can be sampled more than once. Upland geese return to the same territories each season and are remarkably tame on New Island, facilitating repeated measurements of individuals and the monitoring of the reproductive performance of individuals and pairs in consecutive years. We therefore can not only study fitness correlates on a population level but also within individuals, allowing us to calculate repeatability and to make a statement about their role during the evolution of this species. This thesis gives a first insight in the selective forces acting in this species, providing the basis for a long-term study on natural and sexual selection in a non-passerine system.

OUTLINE

In this work we study fitness correlates in wild Upland geese (*Chloephaga picta leucoptera*) and, if applicable, compare them within individuals between years. We thereby aim to identify phenotypic characteristics that are heritable and may have evolved through selection. We will address both aspects of selection, describing traits which most likely underlie natural selection (chapters three to five) and others that could be formed by sexual selection (chapter six and seven).

In **chapter two**, I describe the basic biology of our study species, the Upland goose. I introduce the study location, the New Island Nature Reserve and also give a brief overview over the methods used in the single projects.

In **chapter three** (‘*Timing of breeding and individual condition’*) we show that in female Upland geese annual variation in body condition directly translates into timing of breeding. Females in better condition started to reproduce earlier in a season. Despite this, differences in quality between individuals remained stable as indicated by the significant repeatability of body condition. We can therefore assume that body condition has a heritable component upon which selection can act. Timing of breeding is related to reproductive output with earlier clutches containing more and bigger eggs, and chicks hatched from bigger eggs are in a better initial condition with larger reserves for early development.

Additionally, we here show for the first time that the frequently reported negative relationship between female condition and hatching date also holds true on an intra-individual basis. Although several studies have shown before that waterfowl females in good condition lay larger clutches and start egg-laying earlier in a breeding season, most of these studies lacked corresponding data on individual condition and timing of breeding in different years. These results together with the significant repeatability of body condition may explain how selection shaped timing of breeding in Upland geese.

In **chapter four** (*‘Plasma biochemistry and haematology and parental investment’*) we describe how variations in leucocyte profiles and plasma biochemistry are related to different aspects of parental investment in male and female Upland geese.

In males we found H/L ratio to be related to body condition and capture date, but not repeatable within individuals. Higher H/L ratios of males in a low body condition and later in the year may reflect stress associated with the investment into the establishment and defence of the breeding territory. In male Upland geese, leucocyte profiles and plasma biochemistry appear to be very variable between years as we found none of these parameters to be significantly repeatable between the two consecutive breeding seasons. Together with the result that H/L ratios are related to body condition and time of the season, we can conclude that leucocyte profiles in male Upland geese can be used as a measure of current stress but not as an indicator for the inherent quality of individuals.

In females, leucocyte counts, plasma protein and plasma carotenoid concentrations varied with clutch measures and hatching date in females. Individuals with higher clutch volumes had lower total leucocyte and lymphocyte numbers and higher levels of plasma protein. Earlier hatching dates were associated with lower numbers of all leucocyte types and higher values of plasma carotenoid concentrations. This indicates that differences in health state are reflected in reproductive performance in female Upland geese. The significant repeatability of H/L ratios plasma carotenoid concentrations indicates that these measures might be used as an index for individual quality.

In **chapter five** (*‘Faecal corticosterone metabolites and condition’*) we show that faecal glucocorticoid metabolite (GCM) can be used as a condition measure in wild Upland geese. We found that faecal GCM measures were higher in individuals with lower body condition in both sexes and higher in later breeding females with smaller broods. Additionally, faecal GCM showed a significant repeatability within individuals within a season.

It is well established that baseline plasma corticosterone levels in birds rise with declining body condition, but study so far investigated if this relationship can also be found using faecal samples. Our results suggest that measuring faecal GCM values maybe a valuable non-invasive tool to monitor the relative condition or health of individuals.

The relation of faecal GCM to number of offspring and timing of breeding fits well into the pattern we found for Upland geese namely that both depend mainly on female resources and condition (see chapter two). The earlier hatching dates and larger brood sizes of females with lower CM values may be explained by differences in individual quality. Low quality individuals secrete corticosteroids at higher levels either because they perceive certain environmental circumstances as more challenging per se or because these circumstances are more challenging to them due to their lower condition. As elevated corticosterone levels are assumed to reallocate resources away from reproduction onset of incubation (and thereby hatching date) might be postponed in low quality individuals.

Variation in baseline corticosterone levels has been found to be partly heritable in several avian species with lower quality individuals perceiving their environment as more challenging. The significant repeatability of baseline corticosterone levels in our study supports the use of faecal GCM as indicator of inherent quality in Upland geese.

In **chapter six** (‘*Female-specific colouration’*) we focus on the potential signalling ability of female integument and feather colouration. Although studies on the evolution and function of female ornaments increase in number, there are few studies on female-specific ornaments. We found that female-specific colouration in Upland geese can reliably indicate different aspects of female phenotypic quality. Females with more orange coloured legs and more red-like head colours had higher clutch and egg volumes than females with a paler leg and head colouration, and a more reddish plumage colouration was related to a higher body condition. These relationships provide the theoretic possibility for males to assess female phenotypic quality on the basis of colouration. Furthermore, the females with a more orange-like tarsus colouration had higher plasma carotenoid levels. Both tarsus colouration and carotenoid concentrations of individual females were highly correlated across years, indicating that tarsus colour is a stable signal. Despite this correlation, small individual differences in plasma carotenoid concentrations between the two study years were related to differences in tarsus colouration. We thus show for the first time in a wild bird and under natural conditions, that carotenoid-based integument colouration remains consistent between individuals in consecutive years, but is also a dynamic trait reflecting individual changes in carotenoid levels.

In **chapter seven** (*‘Male achromatic wing colouration’*) we describe the variation in black and white wing colouration. We found that males with darker speculum and a higher contrast between the white wing coverts and the speculum were in a better body condition. However, speculum brightness was not repeatable within individuals, which is in line with our findings for the repeatability of body condition in chapter three, indicating that it may not be a significant predictor for the inherent quality of individuals.

Females mated to males with a darker speculum laid clutches with greater volumes. The relationship of male wing colouration to female reproductive investment could be caused by high quality males in good body condition gaining access to or being able to defend high quality territories, thereby providing the basis for a high investment into clutch size by their female.

The information content of the wing colouration of male Upland geese could be used during mate choice, when females select their mate on the basis of the outcome of aggressive encounters. During these fights males expose the white coverts and the speculum that are normally tucked beneath body feathers.

In **chapter eight** I summarize and discuss our findings.

TWO

The Upland goose
New Island
Methods

THE UPLAND GOOSE

Upland geese *Chloephaga picta*, also called Magellan geese, are a member of the order Tadornini and belong to a group of waterfowl called sheldgeese. Sheldgeese are ecomorphological similar to true geese (Anserinae: Anserini) with an upright stand, a long neck and grazing habit and have been placed in this group by early taxonomists (Livezey 1997). However, these similarities are superficial; looking at certain characters reveals that they are more closely related to shelducks and ducks: different voices in males and females, a bony knob on the bend of the wing that is used during fighting, smooth and glossy eggs and the presence of an iridescent speculum on the greater wing coverts (Summers and McAdam 1993). One of the most interesting aspects in the closely knit group of the genus *Chloephaga* is the great variation in colouration between the species and sexes, ranging from nearly no dimorphism in Andean goose *C. melanoptera* (both sexes mainly white), Ruddy-headed goose *C. rubidiceps* and Ashy-headed goose *C. poliocephala* (both mainly brown plumage), to the Kelp goose *C. hybrida* and the Upland goose exhibiting a very strong sexual dimorphism with males being mainly white and females brown (Summers and McAdam 1993). While this dimorphism is still restricted to the plumage in Kelp geese it even extends to the integument colouration in Upland geese (males: black tarsus, females: yellow-orange tarsus). There is still speculation about the causes for the differences in white colouration in this group, one possibility being that it evolved in order to be conspicuous (Summers and McAdam 1993). Especially Kelp and Upland geese are very aggressive birds, defending large territories and signalling their ownership with erect posturing. A plumage colouration contrasting as much as possible with the background would thus be advantageous. In females, the need to be cryptic during incubation could have preserved the brown plumage.

There are two sub-species of Upland geese, namely the Lesser Magellan goose *C. picta picta*, that can be found on the South American mainland and the Greater Magellan goose *C. picta leucoptera* that is restricted to the Falkland Islands. Upland geese on the Falkland Islands do not show large-scale movements as the populations found on the South American mainland, but rather short ones (usually less than 5 km). First-year birds travel only short distances from their natal area, with males moving slightly further than females. Also the shedding sites are mostly within 5 km of the breeding territory of birds.

Figure 1 Flying pair of Upland geese

Upland geese are highly territorial and socially monogamous, usually returning to the same territory with the same mate every year (Summers and McAdam 1993). Take up of territories starts between August and September, when the pairs, which in most cases stays together throughout the year, return from the winter moulting sites. Male Upland geese invest heavily into the establishment and defence of their breeding territory. They

Figure 2 Male Upland geese defending their territory with increasing aggressiveness from left to right

increase their aggressive activities gradually during the season (Fig.2). Besides evicting their own young from the previous breeding season, the territory has to be defended against intruding pairs looking for a breeding place and territorial boundaries with neighbouring pairs need to be maintained throughout the breeding season (Summers and McAdam 1993). Aggressive encounters range from pacing side by side along the territorial boundaries over the exposure of their carpal knobs to gripping each others by the neck accompanied by the dashing of the wings against each other. When attacking another bird, the male runs rapidly over the ground with its head low and the folded wings slightly spread, thus exposing the white upper coverts and the speculum which are both normally hidden when the wing is closed.

Upland geese are ground-breeders and nests are well hidden in the grass making them hard to spot. Lying starts in late September and most clutches are finished by the end of October. The average clutch consists of 6 eggs. Hatching mainly takes place between mid-October and mid-November, with an average brood size of 5 goslings (Fig. 3). Fledging starts when offspring are about 70 days old and most goslings stay close to their family for the next months to complete their growths under parental protection. Most females are paired by the end of their second year, and it is not fully established, when males breed for the first time. On New Island, we have two observations of breeding males, that were ringed as goslings, one two year old and one three year old (unpubl. data). It can be assumed, that in males the competitive power to establish and defend a breeding territory might be the key to when breeding is started. In Upland geese pair formation takes place in areas where non-breeding birds gather, normally at large greens around ponds that are not held by breeding adults. Females incite males to attack other males and select their mate on the basis of the outcome of these fights (Johnsgard 1965; Summers and McAdam 1993). Once the pair bond is established, Upland geese stay together and divorce is rare. Adult birds have an average life-expectancy of five years, although captive birds have been reported to have reached 14 or even 16 years.

Upland geese on the Falkland Islands have been regarded as a major pest by sheep farmers, who claim that geese compete with sheep for grass. As a reaction large numbers of geese were killed and bounties were paid for beaks (e.g. at least 25,000 geese were killed each year in the late 1970s under farm schemes). However, Summers and McAdam (1993) show, that Upland geese only take about 7-10% of what was eaten by geese, sheep and cattle together of the annual organic matter production of herbage. Additionally, goose droppings are more nutritious than much of the grass, and sheep eat droppings. In the last thirty years there has been a more enlightened attitude towards these geese, and they are no longer persecuted to anything like the same extent, though Upland geese are still classed as pests.

Figure 3 Family of Upland geese

NEW ISLAND

The Falkland Islands are an archipelago in the South Atlantic Ocean, located approximately 460 km from the coast of mainland South America. The archipelago, consisting of East Falkland, West Falkland and 776 lesser islands, is a self-governing British Overseas Territory. New Island is situated in the SW of the Falkland Islands (51°43′S, 61°18′W, middle of the island), the most westerly inhabited place of the island group. It has 84 km of coastline and an area of approximately 2000 ha. It is characterised by steep cliffs on the western side that fall off to the east, where low ground and sheltered bays predominate.

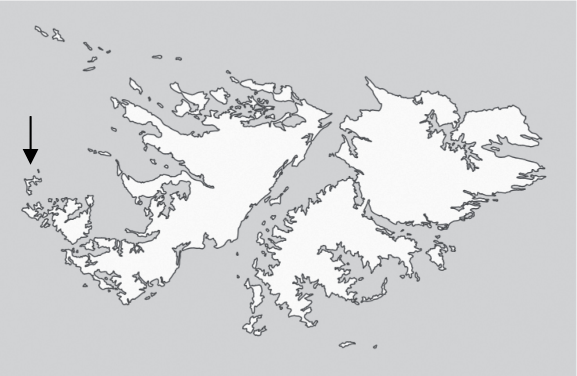

Figure 4 Map of the Falkland Island with New Island (arrow) in the west

New Island has been established as a nature reserve in 1973. Before, it has served as a base for whalers and sealers in from 1700-1800 and been run as a sheep farm for more then 100 years afterwards. As a result, the vegetation suffered from burning and grazing and some areas were covered with introduced grass species. Mammals introduced during this time include mice, rats, cats and rabbits, which still can be found on the island. During the 1970s, when environmental protection started, sheep stock was first reduced to less than half the original number and later removed completely. With the largest seabird colony in the Falkland Islands, New Island is recognized as an Important Bird Area (IBA) by BirdLife International, being a globally important habitat for the conservation of bird populations. It was given official status as a Wildlife Sanctuary under Falkland Islands Government's Sanctuaries and Reserves Ordinance 1964 in 1993. Today New Island is an important conservation site and protected and carefully managed by the New Island Conservation Trust. One of the main goals of the trust is to provide the basis to conduct scientific research on the island, allowing access to the bird colonies, providing accommodation and logistical support. The field station is workspace equipped with laboratory workspace, an office, library and herbarium, as well as with a fully self-contained accommodation for up to 3 or 4 people.

Upland geese are very abundant and widespread on New Island. Counts on the southern part of New Island gave an estimate of between 200 and 400 Upland geese nesting annually on this area, giving a total of about 400-800 breeding pairs. Numbers recorded from flocks of moulting birds later in the year suggest that New Island may harbour moulting geese from other areas of the Falkland Islands. Upland geese seem to benefit from a lack of persecution on New Island as they are remarkably tame, making it easy to work with the birds.

Figure 5 Panorama of the New Island settlement

METHODS

Clutch measurements

At the start of each field season, we mapped nests using Global Positioning System (GPS). Territories were searched until a female flew off or the nest was found by spotting the incubating female. The eggs were covered with down after measurements and all females returned to their nests afterwards. For each nest, we determined clutch size, measured length (L) and breadth (B) and weighed each egg. Egg volume (V) was calculated as $V = (L \times B^2 \times 0.507)$. We defined total clutch volume as the sum of the volumes of all eggs in the clutch.

Capture of birds

Breeding pairs of Upland geese were captured using a whoosh net (3*5m) when leading broods. A whoosh net is a net fired over the birds by strong elastic. The net is furled in a line at the back of the catching area. At either end is a pole stuck in the ground, leaning forward at about 28 degrees. The leading edge of the net is secured at its corners to rings which slide up the poles, and to two lengths of elastic cord. The rings and the net are held at the base of the poles by a trigger mechanism, against the tension of the elastic. When the net is triggered, it flies up the poles, off their ends and over the catching area, then down on to the ground and is held there by the remaining tension in the elastic. One researcher walked the family of geese slowly to the catching area (Fig.6), where the other researcher pulled the trigger once the birds arrived at the required position directly in front of the furled net.

Figure 6 Walking a family of Upland geese to the whoosh net

Adults were marked with an individual metal ring on one leg and a plastic ring readable from a distance on the other. The code on the plastic ring consisted of a letter and a number. Chicks were marked individually using web-tags placed in the foot webs. All birds were weighed and head, culmen, wing (maximum flattened chord) and tarsus length were measured. A blood sample (approximately 300μl) was collected from the brachial vein.

Colour measurements

Digital photography

We measured the colour of the female head and tarsus based on digital photos. We used a Canon EOS 350D digital camera (8.0 megapixels) with a Canon Zoom Lens (EF-S 18–55 mm f/3.5–5.6) with automatic settings for integration time (shutter speed) and lens aperture, and with the white balance set to ‘daylight’. All photos were taken outdoors with the integrated flash lighting. We held the right tarsus next to a yellow reference card close to the ground and took two pictures from above (Fig. 7). Similarly, a photo of the head together with an orange reference card was taken from the left side (Fig. S3). We avoided direct sunlight on tarsus, head and reference card. We imported pictures into Adobe Photoshop CS3, and recorded the red, green

Figure 7 Female tarsus and reference card

and blue (RGB) levels of tarsus, head and the respective reference card using the histogram palette. We analysed the ratio of red to green (R/G) following (Bergman and Beehner 2008). We then used the difference between the tarsus or head R/G value and the respective R/G value of the reference card. To describe what R/G difference values signify in terms of colour, we assessed the colour of our reference card using the GretagMacbeth ColorChecker colour rendition chart [product no. 50105, manufactured by Munsell Colour, division of GretagMacbeth) Higher tarsus R/G values indicate a more orangelike tarsus in our study and lower head R/G values a more red-like head colouration (for a detailed description of the method see chapter six).

Figure 8 GretagMacbeth ColorChecker together with the yellow and orange reference card

Spectrophotometric colour measurements

Spectrophotometric colour measurements were used to measure wing colouration of Upland geese. Reflectance spectra between 300 and 700 nm were recorded using an AvaSpec-2048 Fibre Optic Spectrometer (Avantes, Netherlands) with an AvaLight-DHcDeuterium/Halogen Light Source Unit, and FCR-7UV400 Reflection Probe Fibre optics. We took five readings each from the white head, the white wing coverts and the speculum on each male. As each trait appeared uniform in colour, it was measured in randomly allocated places, moving the probe at least 2 cm between measurements. We used an average reflectance curve from the five readings for each region in the following analyses. Reflection was recorded using a probe held normal to the surface, collecting light from a spot of 4.5 mm in diameter. A white reference (WS-2 white reference tile for reflectance measurements, Avantes, Netherlands) and a dark reference (measured with lights turned off) for calibration were taken before measuring each individual.

Figure 9 Field set-up of spectrophotometer, battery and laptop

Hematology

Blood samples taken from both adults and chicks were used to determine plasma protein, triglyceride and carotenoid levels as well as to conduct a differential leucocyte count examining whole blood air-dry smears. For a detailed description of the method see chapter four.

Hormone sampling

Droppings of Upland geese were collected to determine faecal corticosterone metabolites (CM). Faecal samples were collected when families were leading broods. Sampling took place between 10am and 12am, starting at a mean of 20±1 days after capture of adult birds. Families were approached until a dropping of both the male and female was observed. Individuals were sampled repeatedly (3-6 times) with an interval of 3 to 5 days between sampling sessions. Droppings were collected and kept in plastic tubes on ice–packs in an insulated bag during a sampling session. After returning to the field station (usually within one hour), droppings were processed immediately. To test various sample preservation methods, parts of the samples were air-dried, kept in ethanol and frozen at -20°C. CM concentrations in the samples were later analysed in cooperation with the Veterinary University of Vienna using enzyme immunoassays (EIA).

THREE

Seasonal clutch size decline and individual variation in the timing of breeding are related to female body condition in a non-migratory species, the Upland Goose *Chloephaga picta leucoptera*

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ORIGINAL ARTICLE

Seasonal clutch size decline and individual variation in the timing of breeding are related to female body condition in a non-migratory species, the Upland Goose *Chloephaga picta leucoptera*

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Abstract Several studies have shown that waterfowl females in good condition lay larger clutches and start egg-laying earlier in a breeding season. However, most of these studies lack corresponding data on individual condition and timing of breeding in different years. We analysed data on clutch size, egg size, hatching date and female body condition of a non-migratory species, the Upland Goose *Chloephaga picta leucoptera,* recorded in three seasons on New Island, Falkand Islands. We found a strong seasonal decline in both egg and clutch size. Egg size increased with clutch size. The mean egg volume and total clutch volume increased with female body condition and hatching date was earlier for females in higher body condition. Chicks hatched from nests with higher mean egg volume had higher early body condition. We also compared individual females between different study years and found that individual body condition showed a significant repeatability between years, whereas hatching date did not. While female aging did not systematically affect hatching date, females produced clutches that hatched earlier in years of higher body condition. We could thus show for the first time that the frequently reported negative relationship between female condition and hatching date also holds true on an individual basis. To our knowledge, this is the first study on seasonal clutch size decline in sheldgeese and one of a few in waterfowl comparing condition and timing of breeding within individuals between different years.

Keywords Clutch size decline · Egg size · *Chloephaga picta leucoptera* · Reproductive investment · Body condition

Introduction

There are numerous studies on seasonal variation in reproductive success in birds, one of the most common patterns being a seasonal decline in clutch size (Klomp 1970). Early-nesting birds often lay bigger clutches, and their offspring grow more rapidly and have a higher chance of survival and recruitment than late-nesting birds (Blums et al. 2002; Drent and Daan 1980; Hochachka 1990; Sockman et al. 2006), which results in a decrease in offspring value within a season. In precocial birds such as waterfowl, young receive parental care in the form of vigilance, social support and defence against predators (e.g. Lamprecht 1985; Owen and Black 1990; Schindler and Lamprecht 1987; Winkler and Walters 1983). Parents also lead their offspring to good feeding sites and enable chicks to gain access to these sites when competitors are present (Lepage et al. 1999; Winkler and Walters 1983). However, chicks do not depend on parental feeding, a component that is correlated with clutch size in altricial species (Lack 1968). Instead, the investment into clutch formation plays an important role for precocial chick survival. Most hypotheses concerning clutch size in waterfowl have

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implied that the condition of the female prior to laying is a determinant for reproductive success (Bengston 1971; Johnsgard 1973; Lack 1968; Ryder 1970; Winkler and Walters 1983). The body condition of individuals can vary according to individual quality and experience, leading to increased foraging efficiency, as well as the food resources available in the territory. Thus, high-quality individuals in high-quality territories may gain condition more rapidly after the winter and would therefore be the first to start laying in the breeding season, while low quality individuals in lower quality territories or individuals facing less favourable feeding conditions would lay later (Rowe et al. 1994; Sockman et al. 2006). Drent and Daan (1980) suggested that the conflict between early breeding (high offspring value) and late breeding (higher accumulated condition and potential clutch size) results in the observed timing of laying and clutch size. From a graphical model, Drent and Daan (1980) concluded that birds in poorer condition might have more to gain from a delay than birds in good condition. Field studies have shown that in most species females in good condition lay larger clutches and lay earlier in the season (Klomp 1970; Sockman et al. 2006). Experiments manipulating parental condition by supplementary feeding prior to egg laying resulted in earlier laying and increased clutch sizes (Karell et al. 2008; Meijer et al. 1988, 1990; Rowe et al. 1994).

However, most of the studies on the relationship between body condition and hatching date have been conducted either using data on population-level (e.g. Bety et al. 2003; Dalhaug et al. 1996; Devries et al. 2008; Sjoberg 1994) or, if conducted on an individual basis, then only on repeated observations of clutch size and hatching date without collecting corresponding data on individual condition (e.g. Hamann and Cooke 1987; Petersen 1992). To our knowledge, there is only one study that compares individual females between different years, finding a positive relation between Common Eider *Somateria mollissima* clutch size and female mass, but no relation with hatching date (Erikstad et al. 1993).

Egg size is another important component of parental effort in birds, and several studies have shown a positive relationship between egg size and offspring fitness, in altricial as well as precocial birds. Larger eggs may enhance offspring fitness by increasing survival prospects in the first days after hatching and competitive power through a larger offspring size and the possession of more resources to survive adverse conditions (Amundsen and Stokland 1990; Anderson and Alisauskas 2002; Ankney 1980; Christians 2002; Dawson and Clark 2000; Goth and Evans 2004; Magrath 1992; Potti 1999; Rutkowska and Cichon 2005). Increased egg size through food supplementation could be linked to improved hatchability of the egg, increased size at hatching, early growth and survival during the nestling stage (reviewed in Williams 1994; Christians 2002, see also Grindstaff et al. 2005).

We studied seasonal egg and clutch size decline and their relation to female body condition in a non-migratory waterfowl species, the Upland Goose *Chloephaga picta leucoptera*. Upland Geese belong to the order of the sheldgeese (Tadornini), a group that physically resembles true geese (Anserinae) and shows similar habits but is more closely related to shelducks and ducks. The smaller (migratory) sub-species *C. picta picta* breeds on the South American mainland, whereas the slightly larger one (*C. picta leucoptera*) is restricted to the Falkland Islands. Their basic breeding biology and life-cycle has been studied in the Falklands from 1977 to 1980 (Summers 1983). Upland geese are highly territorial and socially monogamous, usually returning to the same territory with the same mate every year. Territories are taken up from August onwards and eggs laid between late September and end of October. The mean clutch comprises 6 eggs (Summers and McAdam 1993). Hatching mainly takes place between mid-October and mid-November and fledging starts at about 70 days of age. No creching occurs during the breeding season, and family groups stay together for the austral summer and winter (Summers 1983).

Additionally to the general analysis of the relationship of clutch size, hatching date and body condition in this non-migratory waterfowl species, we were able to compare data from individual females captured in subsequent years in the same territories. To our knowledge, this is the first study on seasonal clutch size decline in sheldgeese, and one of a few comparing condition and timing of breeding within individuals between different years in waterfowl.

We tested the following hypotheses:

1. Egg and clutch size decrease with increasing hatching date.
2. Egg size, clutch size and hatching date is related to female body condition.
3. Individual variation in hatching dates is related to individual variation in body condition.
4. Chicks hatched from larger eggs are in a better condition than chicks from smaller eggs.

Methods

The study was carried out in the New Island Nature Reserve, Falkland Islands (51°43′S, 61°17′W) from October to December in 2005, 2007 and 2008. New Island has an estimated 84 km of coastline embracing some 2,300 ha. The island has been established as a nature reserve in 1970 when all livestock was removed from the island. This led to an increase in the density of Upland Geese, which is now, with approximately 0.8 pairs ha^{-1}, one of the highest in the Falkland Islands (Quillfeldt et al. 2005).

For the study of seasonal decline, a total of 84 nests were included in the analysis (29 from 2005, 25 from 2007 and 30 from 2008). At the start of each field season, we mapped nests using GPS. Females left their nests at varying distances when approached, eggs were covered with down after measurements and all females returned to their nests afterwards. For each nest, we determined clutch size, measured length (L, expressed in cm) and width (B, expressed in cm) of each egg to the nearest 0.1 mm using callipers, and weighed each egg to the nearest 0.1 g using a digital balance. Egg volume (V, in cm^3) was calculated as $V = (L \times B^2 \times 0.507)$ following Furness and Furness (1981). We defined total clutch volume as the sum of the volumes of each egg in the clutch. Avian eggs decrease in density with increasing length of incubation mainly due to water loss. In 2005, a subsample of eggs was reweighed at a later date to estimate the density loss. Eggs lost an average of 0.0063 ± 0.0005 SE g/cm^3 per day. We determined expected hatching date as follows: (1) we calculated egg density (D) from egg mass M (expressed in g) and V; (2) our 2005 data showed that hatching occurred at a mean egg density of 0.89 g/cm^3 ± 0.01 g/cm^3 SE ($n = 14$ eggs), and (3) the number of days to hatching T was thus estimated as $T = (D - 0.89 \text{ g/cm}^3) / 0.0063 \text{ g/cm}^3$. We then visited nests again at least once a day, starting at the estimated hatching date. All eggs hatched within 0–2 days of the estimated hatch date.

We caught adults during the period when they attended their brood (mean chick age: 14.8 ± 4.1 days) using a 3 × 5 m whoosh net. One person herded the family of geese slowly to the catching area, and when they arrived directly in front of the furled net, the other researcher pulled the trigger. Adults were marked with an individual metal ring on one leg and a plastic ring readable from a distance on the other, and weighed to the nearest 10 g using a digital spring balance. Head length, culmen length and tarsus length were measured to the nearest 0.1 mm using callipers, and wing length (maximum flattened chord) was measured to the nearest 1 mm using a foot rule. Chicks were marked individually using web-tags placed in the foot webs. We left enough space for the foot web to grow and did not observe any adverse effects on gosling growth. Chicks were weighed to the nearest 1 g using a spring balance (<300 g) or to the nearest 10 g using a digital spring balance (>300 g). We measured head length, culmen length, wing length and tarsus length (±0.1 mm) using callipers. Chick age was determined from a growth curve for head and tarsus lengths established from chicks of known age in 2005. For each clutch, the mean chick age and thereby mean hatching date were calculated. As hatching dates could not be determined from egg density for all individual females, we used this estimated hatching date for further analyses. Estimated hatching dates from egg measures and chick measures were highly correlated ($R = 0.973$, $P < 0.001$, $n = 41$).

The body condition of females was then determined accounting for structural size and chick age, as we did not catch females during the prelaying period and captured females had goslings of different age. Females lose weight during incubation, and regain weight after hatching of the chicks (Summers and McAdam 1993). We estimated an expected body mass for each individual based on a multiple linear regression of body mass on the first principal component (PC1) of measurements of wing, head, bill and tarsus and chick age. Body condition was calculated as the ratio of the observed body mass to the derived expected body mass, according to female size and chick age. For the analysis of the effect of mean egg volume on chick body condition, we only included clutches with chicks ≤20 days of age as until then no sex-specific growth was observed (A. Gladbach, unpublished data). Chick body condition was expressed as the ratio of observed to expected body mass. To avoid using the same data to determine chick body condition and chick age (see above), in this case we used the estimated hatching date determined from egg density data. Expected body mass was the population mean of chicks of the same age (based on body mass data of a total of 553 chicks), following Quillfeldt (2002). Using this method, age is included indirectly into the model and hence controlled for. As a result, body condition is by definition uncorrelated (orthogonal) to age. For each clutch, we calculated a mean body condition of chicks.

Statistical analysis

Statistical tests were performed in SPSS 11.0 and R 2.9.1 (R Development Core Team 2009, http://www.r-project.org). Normality was tested with Kolmogorov–Smirnov tests and data were transformed, if necessary. Means are given with standard errors. We assessed significance using Pearson correlations or F statistics in General Linear Models (GLM), based on Type III Sum of Squares. To indicate the direction (positive or negative) of the correlation to the covariable, we included t values, and as a measure of effect sizes, we included partial eta-squared values (η^2) (i.e. the proportion of the effect + error variance that is attributable to the effect). The sums of the partial eta-squared values are not additive (e.g. http://web.uccs.edu/lbecker/SPSS/glm_effectsize.htm). To analyse the dependency of mean egg volume on clutch size, we used a non-parametric Spearman correlation.

For the analysis of individual data from different years, we used a linear mixed effects model (lme) in R 2.9.1 with hatching date as the response variable, body condition and year (as ordered fixed factor) as explanatory variables and female identity as a random effect. In this way, we could simultaneously test whether hatching date on an individual basis depended on body condition or if there was a relation with increasing age (year). In the final model, "year" was excluded based on the stepwise AIC function, an information-theoretical approach (Burnham and Anderson 2004). We present here results of this final model.

Statistical repeatabilities of variables in different years were calculated by the method of Lessells and Boag (1987). Significance level was set to $P < 0.05$. Some territories were not visited before chicks hatched and hence some data on clutch size and egg volumes were missing, resulting in different sample sizes for the respective tests.

Results

Egg and clutch size decline

Clutch size and mean egg volume were significantly higher in clutches with earlier hatching date (Fig. 1; Table 1). Mean egg volume increased significantly with increasing clutch size (Pearson correlation, $R = 0.377$, $P < 0.001$). This correlation remained significant when controlling for female structural size ($R = 0.373$, $P = 0.018$), but not when controlling for female mass ($R = 0.221$, $P = 0.171$) or body condition ($R = 0.238$, $P = 0.139$).

Female body condition and hatching date, clutch size and mean egg volume

Hatching date and female post-hatching body condition were positively related, with females in a better body condition having earlier clutches (Fig. 2; Table 2).

We compared data of 28 recaptured individuals (5 captured in all three study years, 4 in 2005 and 2008, 4 in 2005 and 2007 and 15 individuals in 2007 and 2008). Body condition data showed significant repeatability ($r = 0.308$, $F_{27,60} = 1.969$, $P = 0.032$), whereas repeatability of hatching date was non-significant ($r = -0.171$, $F_{27,60} = 0.683$, $P = 0.844$). Hatching date was significantly negatively related to body condition within individuals (lme in R; body condition: $F_{1,32} = 18.80, t = -4.336, P \leq 0.001$), while changes in year (i.e. female age) did not systematically affect hatching date as it was dropped from the full model (see "Statistical analysis").

Clutch size and mean egg volume were higher in females in better post-hatching body condition and this relationship was independent of the sample year (Fig. 3; Table 2).

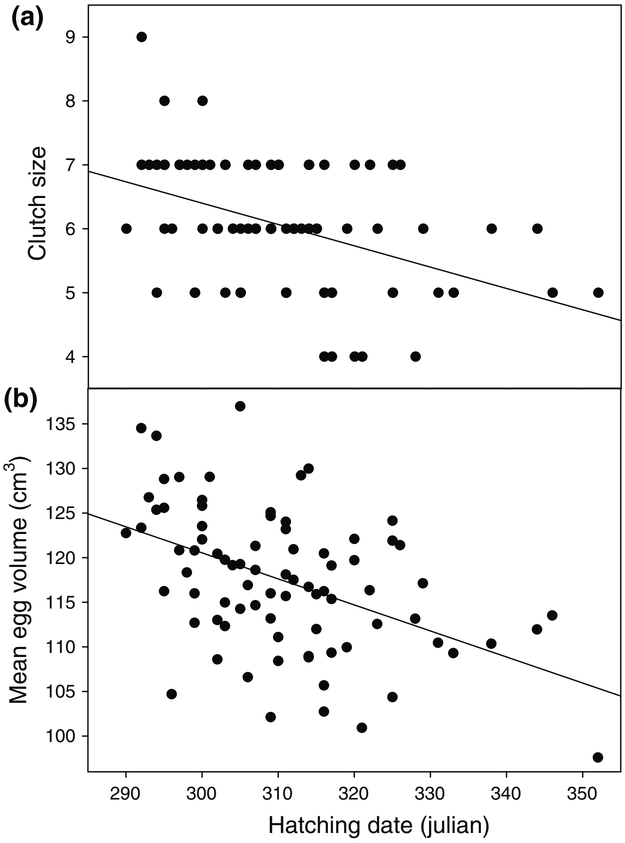

Fig. 1 Seasonal decline of clutch size (**a**), and mean egg volume (**b**) in Upland Geese *Chloephaga picta leucoptera* on New Island

Egg volume and chick body condition

Chick body condition was positively related to mean egg volume and negatively to hatching date, i.e. chicks hatched from nests with higher mean egg volume and earlier clutches had significantly higher early body condition (Table 3; Fig. 4). Clutch size had no effect on chick early body condition (Table 3). Mean body condition of chicks from females with high body condition was higher compared to chicks of low body condition females (Table 3).

Discussion

In our present study on Upland Geese we found that:

1. Clutch size and egg size decreased with later hatching date.
2. Clutch size variables and hatching date were related to female post-hatching body condition.
3. Individual variation in hatching dates was related to individual variation in body condition.
4. Egg size had a direct and positive effect on chick body condition in the first weeks after hatching.

Table 1 Results of general linear models (GLM) showing the effects of year and hatching date on clutch size and mean egg volume in adult female Upland Geese *Chloephaga picta leucoptera*

Parameter	Year	Hatching date (hd)	Interaction (year × hd)
Clutch size	$F_{2,84} = 0.469$, $P = 0.628$, $\eta^2 = 0.012$	$F_{1,84} = 13.213$, $\mathbf{P < 0.001}$, $\eta^2 = 0.145$, $t = -1.300$	$F_{2,84} = 0.434$, $P = 0.649$, $\eta^2 = 0.011$
Mean egg volume	$F_{2,84} = 1.505$, $P = 0.228$, $\eta^2 = 0.037$	$F_{1,84} = 20.183$, $\mathbf{P < 0.001}$, $\eta^2 = 0.206$, $t = -1.273$	$F_{2,84} = 1.528$, $P = 0.223$, $\eta^2 = 0.038$

Clutch size or mean egg volume were used as dependent, year as a fixed factor and hatching date as covariate. η^2 was included to indicate the effect size and t values to indicate the direction (positive or negative) of the correlation to the covariable. Significant P values are shown in bold

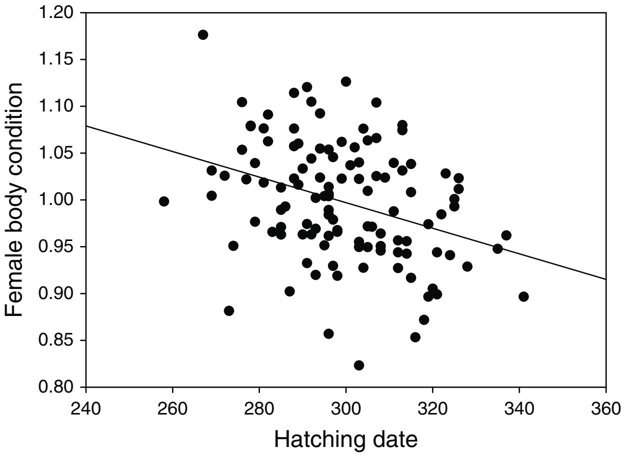

Fig. 2 Negative relationship between female body condition and hatching date in Upland Geese

We found the observed seasonal decline of clutch and egg size on the population level in Upland Geese to be mainly caused by the early onset of reproduction by females in good body condition. We could show, to our knowledge for the first time, that the commonly observed positive relation between female condition and hatching date also holds true on an individual basis. Annual variation in individual body condition had a direct effect on the onset of reproduction, with females in an individually better condition having earlier hatching dates. The significant repeatability of post-hatching body condition indicates that differences in quality between individuals remain stable over several years, but that despite this stability small individual changes in body condition affect timing of reproduction. However, as we did not measure female pre-breeding condition, we are aware that our results must be treated cautiously as it may not necessarily be linked to her post-hatching condition, an assumption that still needs to be tested in Upland Geese.

Differences in body condition between individuals may arise due to differences in the inherent quality of individual birds (e.g. Jensen et al. 2003; e.g. Merila et al. 2001), which is supported by the significant repeatability of body condition in our study. As Upland Geese return to the same territories every year, also differences in territory quality may affect female body condition. Differences in individual body condition could have more immediate causes such as differences in weather, food availability, health state etc. That small differences in condition affect the timing of the onset of reproduction supports the idea that (1) egg laying starts when a certain individual threshold body condition is reached, and (2) that the investment into egg size and number decreases as the season advances, as expected offspring value is assumed to decline during a breeding season. Alternatively, if female-post-hatching condition is closely linked to her pre-laying condition, the investment into more and heavier eggs could also be explained by this better body condition. In contrast to previous studies that found an age effect with earlier hatching dates in older age classes to be the main cause for inter-individual variation in hatching dates (Blums et al. 2002; Finney and Cooke 1978;

Table 2 Results of general linear models (GLM) showing the effects of year and body condition on hatching date, clutch size and mean egg volume in adult female Upland Geese

Parameter	Year	Body condition (bc)	Interaction (year × bc)
Hatching date	$F_{2,83} = 0.301$, $P = 0.741$, $\eta^2 = 0.008$	$F_{1,83} = 10.138$, $\mathbf{P = 0.002}$, $\eta^2 = 0.116$, $t = -1.199$	$F_{2,83} = 0.303$, $P = 0.739$, $\eta^2 = 0.008$
Clutch size	$F_{2,41} = 1.314$, $P = 0.282$, $\eta^2 = 0.070$	$F_{1,41} = 10.741$, $\mathbf{P = 0.002}$, $\eta^2 = 0.235$, $t = 3.174$	$F_{2,41} = 1.119$, $P = 0.338$, $\eta^2 = 0.060$
Mean egg volume	$F_{2,39} = 0.356$, $P = 0.703$, $\eta^2 = 0.021$	$F_{1,39} = 15.366$, $\mathbf{P < 0.001}$, $\eta^2 = 0.318$, $t = 2.085$	$F_{2,39} = 0.503$, $P = 0.609$, $\eta^2 = 0.030$

Hatching date, clutch size or mean egg volume were used as dependent, year as a fixed factor and hatching date as covariate. η^2 was included to indicate the effect size and t values to indicate the direction (positive or negative) of the correlation to the covariable. Significant P values are shown in bold

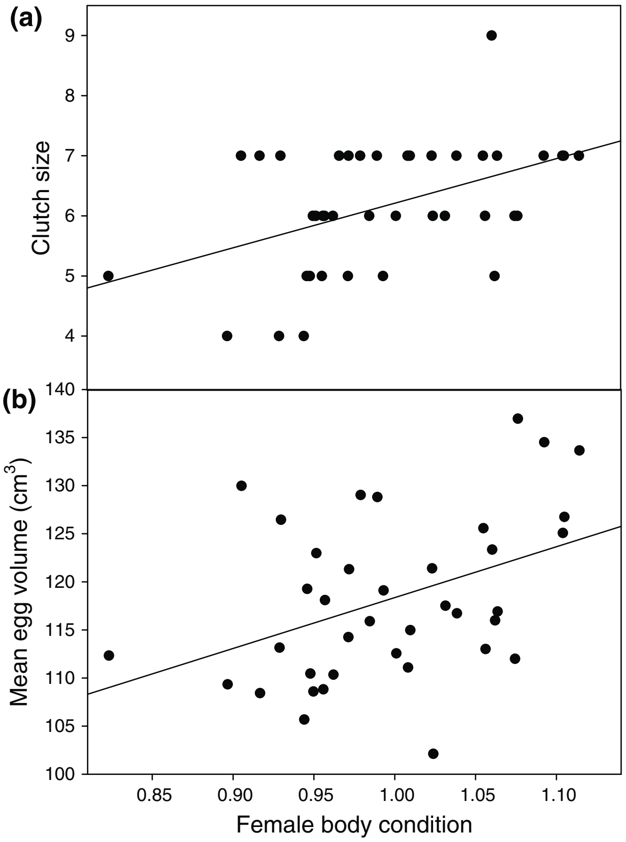

Fig. 3 Positive relationship between female body condition and clutch size (**a**), and mean egg volume (**b**) in Upland Geese on New Island

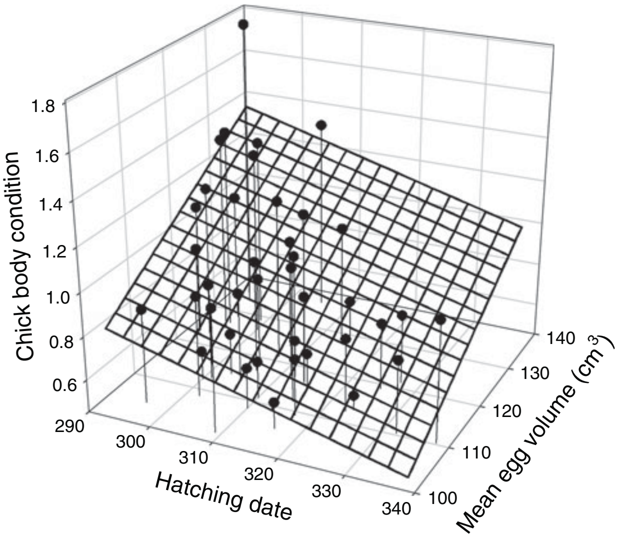

Fig. 4 Relationship between chick body condition, hatching date and mean egg volume in Upland Geese

Hamann and Cooke 1987, 1989), we did not observe a decline in hatching date within individual females over the study years. Upland Geese have an average adult life expectancy of about 5 years (Summers and McAdam 1993),so our study period covered a considerable amount of this expected lifespan. However, the small sample size of individuals sampled in three different years ($n = 5$) may also explain why we could not detect an age effect in our study. One hint of the possibility of an age effect at the very end of an individual life span is one female found dead at the end of the last study year which had hatched chicks 30 days earlier in 2008 than in 2005 and 2007.

In precocial species like waterfowl, where the young feed for themselves after hatching, the role of the parents and their effect on offspring performance is harder to detect than in altricial species. Parental quality may not only be reflected in the effect of social factors like predator avoidance/defence and social competence for variation of reproductive success. High quality parents may also be able to produce larger clutches and to accumulate more resources more rapidly than low quality individuals which

Table 3 Results of general linear models (GLM) showing the effects of year, hatching date and (*A*) mean egg volume, (*B*) clutch size and (*C*) female body condition on early chick body condition in Upland Geese

A	Year	$F_{2,39} = 0.844$, $P = 0.439$, $\eta^2 = 0.047$
	Hatching date	$F_{1,39} = 4.582$, $\mathbf{P = 0.040}$, $\eta^2 = 0.119$, $t = -2.141$
	Mean egg volume	$F_{1,39} = 12.904$, $\mathbf{P = 0.001}$, $\eta^2 = 0.275$, $t = 3.592$
B	Year	$F_{2,39} = 0.022$, $P = 0.978$, $\eta^2 = 0.001$
	Hatching date	$F_{1,39} = 5.547$, $\mathbf{P = 0.024}$, $\eta^2 = 0.140$, $t = -2.355$
	Clutch size	$F_{1,39} = 4.023$, $P = 0.053$, $\eta^2 = 0.106$, $t = 2.006$
C	Year	$F_{2,39} = 2.802$, $P = 0.075$, $\eta^2 = 0.141$
	Hatching date	$F_{1,39} = 3.531$, $P = 0.069$, $\eta^2 = 0.094$, $t = -1.879$
	Female body condition	$F_{1,39} = 2.597$, $\mathbf{P < 0.001}$, η^2 535 $= 0.420$, $t = 4.960$

Chick body condition was used as dependent, year as a fixed factor and hatching date and mean egg volume, clutch size or female body condition as covariates. η^2 was included to indicate the effect size and t values to indicate the direction (positive or negative) of the correlation to the covariable. Significant P values are shown in bold would allow an earlier nesting (Drent and Daan 1980) and thereby providing better growing conditions for the offspring (Hoekman et al. 2004; Lepage et al. 1998, 1999). Female egg investment may play an important role in determining survival prospects of offspring (Amat et al. 2001) and thereby increasing individual fitness. We here show that early chick body condition increased with increasing egg size. This is line with other studies on waterfowl, where chick body size was also found to be related to egg size (e.g. Christians 2002; Ankney 1980; Anderson and Alisauskas 2002).

Interestingly, we found that egg size increased with increasing clutch size. Other studies on waterfowl have already shown that the theoretical trade-off between size and number of offspring as proposed by Lack (1968) can be observed on an interspecific level (Figuerola and Green 2006), but is not always present on an intraspecific level (Christians 2000; see Christians 2002 for a review). The intraspecific relationship does not show a consistent pattern, as there was no correlation between egg size and clutch size in some species but a positive correlation in others (see Christians 2002 for a review). Hõrák et al. (2008) pointed out that the absence of a trade-off between egg size and number may be explained by the lower quality of young hatched from larger clutches, i.e. the trade-off occurring between egg number and hatchling quality. We found no evidence that this is true for Upland Geese, as clutch size had no influence on chick body condition.

Flint et al. (1996) suggested that, in waterfowl which rely on endogenous reserves for egg production, large-bodied birds may tend to lay larger c1utches of larger eggs than do small-bodied birds. Our results show that in Upland Geese body size is no proxy for egg size as we failed to detect a relationship between structural size and either egg or clutch size. In contrast, the availability of endogenous reserves (reflected in body mass and body condition) was an important source for variation in egg and clutch size between female Upland Geese. This supports the idea that high-quality females are able to produce more and larger eggs than low-quality females, although we could not distinguish whether the higher body condition of these females was caused by higher foraging efficiency, or indirectly by defending a high-quality territory. Assessing the body condition during the non-breeding season, when Upland Geese gather in large groups around ponds, could shed further light on the importance of the breeding territory or winter condition for the differences in body condition during the breeding season. In addition, a comparison of individual clutch and egg size measures in relation to body condition over several years, which was not possible in this study, could further deepen our understanding of optimal investment.

Zusammenfassung

Saisonaler Rückgang der Gelegegröße und individuelle Variation des Brutgbeginns sind bei der nicht-ziehenden Magellangans *Chloephaga picta leucoptera* von der weiblichen Körperkondition abhängig

Viele Studien an Wasservögeln haben gezeigt, dass Weibchen in guter Kondition größere Gelege haben und früher in der Brutsaison mit der Eiablage beginnen. Allerdings fehlen in diesen Studien parallele Daten über individuelle Kondition und Brutbeginn in verschiedenen Jahren. Wir haben Daten über die Gelege- und Eigröße, das Schlupfdatum und die Körperkondition der Weibchen bei der Magellangans *Chloephaga picta leucoptera* während dreier Brutsaisons auf New Island, Falklandinseln, aufgenommen und untersucht, inwieweit Variabilität von Reproduktionsparametern und Körperkondition sowohl innerhalb als auch zwischen Weibchen zusammenhängen.

Wir fanden eine starke saisonale Abnahme von sowohl Ei- als auch Gelegegröße auf Populationsebene. Ei- und Gelegegröße nahmen mit steigender Weibchenkondition zu und Weibchen in besserer Körperkondition hatten frühere Gelege. Küken aus Gelegen mit größeren Eiern waren in einer besseren Körperkondition. Der Vergleich individueller Daten aus verschiedenen Jahren ergab dass die Körperkondition zwischen den Jahren signifikant wiederholbar war, das Schlupfdatum jedoch nicht. Während Unterschiede im Alter der Weibchen keinen Effekt auf das Schlupfdatum hatten, ging eine individuelle Verbesserung der Kondition mit einem früheren Schlupfdatum einher. Wir konnten damit zum ersten Mal zeigen, dass der bekannte allgemeine Zusammenhang von Körperkondition der Weibchen und Reproduktionszeitpunkt auch auf individueller Ebene Gültigkeit besitzt. Unserem Wissen nach ist dies die erste Studie zu saisonaler Gelegeverkleinerung bei Halbgänsen und eine von wenigen an Wasservögeln die Körperkondition und Reproduktionszeitpunkt innerhalb von Individuen und zwischen verschiedenen Jahren vergleicht.

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FOUR

Variations in leucocyte profiles and plasma biochemistry are related to different aspects of parental investment in male and female Upland geese *Chloephaga picta leucoptera*

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Variations in leucocyte profiles and plasma biochemistry are related to different aspects of parental investment in male and female Upland geese *Chloephaga picta leucoptera*

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ABSTRACT

The analysis of plasma biochemistry and haematology to monitor the condition of birds in the wild has been found a useful tool in ecological research. Despite biparental investment in most wild birds studied, some studies of condition indices found sex differences, and attributed these to the costs of egg formation or brooding in females or a higher contribution of males to chick rearing. We studied the natural variation of haematological and plasma biochemistry parameters (namely leucocyte, lymphocyte and heterophil counts, H/L ratio and plasma concentrations of proteins, triglycerides and carotenoids) in relation to the different measures of parental investment in males and females in the Upland goose (*Chloephaga picta leucoptera*), a socially monogamous species. We found no sex differences in haematological and most plasma biochemistry parameters, but a relation to different aspects of parental investment in breeding male and female Upland geese. H/L ratios were related to body condition and capture date in males while leucocyte counts, plasma protein and plasma carotenoid concentrations varied with clutch measures and hatching date in females. Higher H/L ratios of males in a low body condition and later in the year may reflect stress associated with the investment into the establishment and defence of the breeding territory. Females with higher clutch volumes had lower total leucocyte and lymphocyte numbers and higher levels of plasma protein. Earlier hatching dates were associated with lower numbers of all leucocyte types and higher values of plasma carotenoid concentrations. This indicates that differences in health state are reflected in reproductive performance in female Upland geese. We also found sexual differences in the repeatability of haematological and plasma biochemistry parameters between years and therefore suggest that their potential as a measure of individual quality differs between male and female Upland geese. Finally, numbers of leucocyte counts and plasma triglyceride concentrations of pair partners were significantly related. No study so far investigated these parameters in pair partners and we discuss possible reasons for our finding.

1. Introduction

Many studies assessing the relationship of condition indices and reproductive performance in wild birds have been carried out in species, where males and females share a great amount of the duties associated with chick rearing and territory defence (e.g. Kilgas et al., 2006b; Moreno et al., 1998; e.g. Moreno et al., 2002b; Shutler et al., 2004). Despite the biparental care some of the studies found sex differences in stress measures, and attributed this to the costs of egg formation (Jakubas et al., 2008; Kilgas et al., 2006a; Moreno et al., 2002b) or brooding (Hõrak et al., 1998a) in females or a higher contribution of males in the late phase of chick rearing (Jakubas et al., 2008).

Our study describes the natural variation of haematological and plasma biochemistry parameters (namely leucocyte, lymphocyte and heterophil counts, H/L ratio and plasma concentrations of proteins, triglycerides and carotenoids) in relation to the different measures of parental investment in males and females such as incubation and chick rearing in the Upland goose (*Chloephaga picta leucoptera*). Upland geese belong to the order of the sheldgeese (Tadornini), a group that resembles true geese and shows similar habits, but is more closely related to shelducks and ducks. The basic breeding biology and life-cycle of Upland geese has been studied in the Falklands from 1977 to 1980 (Summers, 1983). Upland geese are highly territorial and socially monogamous, usually returning to the same territory with the same mate every year. Take up of territories starts in August, egg laying commences in late September, and most clutches are finished by the end of October. Hatching mainly takes place between mid-October and mid-November and fledging starts when chicks are about 70 days old. Males and females differ in their specific parental roles, with males establishing and intensely defending the territory and females incubating and brooding. We predict that these differences in parental roles are reflected in haematological and blood chemistry parameters.

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The analysis of plasma biochemistry and haematology to monitor the condition of birds in the wild has been found a useful tool in ecological research, as it may give a more integrative picture of the state of an animal than condition indices based on body mass alone. Leucocyte profiles, i.e. the relative numbers of different leucocyte types in the peripheral blood, have been used to study the health and condition of birds in the wild (reviewed in Davis et al., 2008). Lymphocytes and heterophils make up the majority of white blood cells in birds. Lymphocytes are highly specific and involved in a variety of immunological functions like the modulation of the immune response and immunoglobulin production (Campbell, 1995; Hawkey and Dennet, 1989). They increase in numbers during parasitic infection (Bonier et al., 2007; Ots and Hõrak, 1998) and immunological challenges (Eeva et al., 2005). A decrease in lymphocyte numbers may either be linked to stress-induced immunosuppression (Hõrak et al., 1998b) or the absence of parasite infections. Heterophils are non-specific phagocytic cells that proliferate in response to infections, inflammation and stress (Campbell, 1995; Maxwell and Robertson, 1998) and increased numbers can be found during stress, trauma and chronic bacterial infections. The heterophil/lymphocyte ratio (H/L) is often used as stress indicator in birds (Gross and Siegel, 1983; Maxwell, 1993), that is known to increase in the presence of various stressors, such as infectious diseases or starvation.

Some simple measures of blood chemistry in relation to health and condition of individuals include plasma protein concentrations, which are considered to be linked to nutritional status in birds, with rising concentrations when dietary protein intake increases or depressed levels indicating nutritional inadequacies (Jenni-Eiermann and Jenni, 1996; Jenni-Eiermann and Jenni, 1997, 1998; Ots et al., 1998; Rodríguez et al., 2005). High values of protein may also be caused by hemoconcentration, e.g. due to dehydration. Plasma triglycerides have also been linked to health state and fat reserves (Lloyd and Gibson, 2006; Masello and Quillfeldt, 2004; Quillfeldt et al., 2004), as they reflect the deposition of lipids into adipose tissues and thereby recent nutritional changes. Fasting individuals in a post-resorptive state, where triglycerides are hydrolysed from adipose tissues generally exhibit lower values of triglycerides (e.g. Jenni-Eiermann and Jenni, 1997; Jenni-Eiermann and Jenni, 1998). A third indicator of health state is the plasma concentration of carotenoids. Carotenoids are natural pigments that cannot be synthesized by vertebrates and hence must be obtained via the diet (Brush, 1981; Fox, 1979; McGraw, 2005). They have a range of health-related functions and are known to work as antioxidants and immune-enhancers (Lozano, 1994; Olson and Owens, 1998) and both direct (plasma concentration) and indirect (plasma hue) measures of carotenoids could be linked to body condition (Mougeot et al., 2009), immunocompetence (Mougeot et al., 2007; Perez-Rodriguez et al., 2008b; Quillfeldt et al., 2004) and ornamentation (Masello and Quillfeldt, 2004; Mougeot et al., 2009).

The specific aims of the current study were

1. to determine possible sex differences in haematological and plasma biochemistry parameters in Upland geese,
2. to determine whether haematological and plasma biochemistry parameters are linked to sex-specific parental investment,
3. to estimate repeatability within individuals in consecutive years and to determine possible influences on variability, and
4. to compare haematological and plasma biochemistry parameters between pair members of Upland geese.

2. Materials and methods

2.1. Study site

The study was carried out in the New Island Nature Reserve, Falkland Islands (51°43′S, 61°17′W) from October to December 2007 and 2008. The island has been established as a nature reserve in 1970 when all livestock was removed from the island. This led to an increase in the density of Upland geese, which is now one of the highest in the Falkland Islands (Quillfeldt et al., 2005).

2.2. Field measurements and sampling

At the start of each field season we mapped nests using GPS. For each nest, we determined clutch size, measured length (L, expressed in cm) and breadth (B, expressed in cm) of each egg to the nearest 0.1 mm using callipers and weighed each egg to the nearest 0.1 g using a digital balance. Egg volume (V; in cm^3) was calculated as $V=(L\times B^2\times 0.507)$ following Furness and Furness (1981). We defined total clutch volume as the sum of the volumes of each egg in the clutch. Avian eggs decrease in density with increasing length of incubation mainly due to water loss. We estimated density loss during a preliminary study in 2005 by weighing 41 eggs (from 6 clutches) twice within a period of 3–10 days. Eggs lost an average of 0.0063 ± 0.0005 SE g/cm^3 per day. We determined expected hatching date as follows: (1) we calculated egg density (D) from egg mass M (expressed in g) and V as $D=M/V$. (2) Our 2005 data showed that hatching occurred at a mean egg density of 0.89 g/cm$^3\pm 0.01$ g/cm^3 SE ($n=14$ eggs). (3) Thus, the number of days to hatching T was estimated as $T=(D-0.89\text{ g/cm}^3)/0.0063\text{ g/cm}^3$. We visited nests at least once a day, starting at the estimated hatching date; all eggs hatched within 0–2 days from the estimated hatch date.

We caught adults during the period when they attended their brood (chick ages 0 to 45 days, mean 11 ± 1.4 days) using a 3×5 m whoosh net. One person herded the family of geese slowly to the catching area, and when they arrived directly in front of the furled net, the other researcher pulled the trigger. Adults were marked with individual metal rings and weighed to the nearest 10 g using a digital spring balance. Head length, culmen length, and tarsus length were measured to the nearest 0.1 mm using callipers; wing length (maximum flattened chord) was measured to the nearest 1 mm using a foot rule. A blood sample (approximately 300 µL) was collected from the brachial vein. Blood samples were kept cold (4 °C) and centrifuged within 4 h. The separated plasma was stored at −20 °C until plasma protein, triglyceride and carotenoid levels were determined (see below).

Chicks were marked individually using web-tags and weighed to the nearest 1 g using a spring balance (<300 g) or to the nearest 10 g using a digital spring balance (>300 g). We measured head length, culmen length, wing length and tarsus length (±0.1 mm) using callipers. Chick ages were determined from a growth curve for head and tarsus established from chicks of known age in 2005. For each clutch, the mean chick age and thereby mean hatching date were calculated. As hatching dates could not be determined from egg density for all pairs, we used this estimated hatching date for further analyses. Estimated hatching dates from egg measures and chick measures were highly correlated ($r=0.973$, $P<0.001$, $N=41$).

The body condition of adults was determined accounting for structural size based on a regression of body mass on the first principal component (PC1) of measurements of wing, head, bill and tarsus. Body condition was then calculated as the ratio of the observed body mass to the derived expected body mass.

2.3. Leucocyte counts

The differential leucocyte count was determined as described by Ruiz et al. (2002) by examining whole blood air-dry smears. Immediately after returning from the field (no later than 4 h after sampling), the blood sample was well shaken and a drop of blood was smeared on a glass slide, using the standard two slide wedge procedure. All samples were fixed with absolute methanol. In the laboratory, smears were stained using Giemsa stain at a 1:10 dilution, for 20 min. Differential leucocyte counts were carried out with a light microscope (1000×, magnification with oil immersion), crossing the sample from down to up to minimize differences in the thickness of the blood smear. The differential count included relative percentages of lymphocytes (L), heterophils (H), monocytes, basophils and eosinophils, which were identified according to the criteria of Hawkey and Dennet (1989). A total of 100 leucocytes were counted per slide. Using the percentages of heterophils and lymphocytes, the H/L ratio was determined. The number of leucocytes per 10,000 erythrocytes was calculated by counting the number of erythrocytes per field and multiplying by the number of fields viewed to count 100 leucocytes (e.g. Lobato et al., 2005; Merino et al., 1999; Moreno et al., 2002a).

2.4. Plasma protein, triglycerides and carotenoids

Plasma protein and triglycerides were determined using standard spectrophotometric test combinations modified for small amounts of plasma (6 µL plasma per determination, Thermo Fisher Scientific Clinical Diagnostics).

We quantified plasma carotenoid levels following Alonso-Alvarez et al. (2004). We diluted 10 µL of plasma in 90 µL of absolute ethanol, vortexed the mixture and centrifuged it at 1500 g to precipitate flocculent proteins. The supernatant was examined in an Ultrospec 2000 (Pharmarcia Biotech) spectrophotometer and the optical density at 450 nm (maximal absorbance of lutein) was determined. We calculated plasma carotenoid concentration (µg/ml) using a standard curve of lutein (Sigma Chemicals). Each plasma sample was double-tested and the obtained values were highly correlated ($r = 0.97$, $n = 83$, $P < 0.001$). Alonso-Alvarez et al. (2004) assessed the reliability of this technique by comparing a HPLC analysis of plasma samples with colorimetric measurements. HPLC determined the presence of four carotenoids in the plasma (lutein, zeaxanthin, anhydrolutein, and β-cryptoxanthin). The total amounts of carotenoids determined by the two techniques were highly correlated; colorimetric measurements could thus be considered as representative of total plasma carotenoid concentration.

2.5. Statistical analysis

We captured and measured 34 females and 33 males in 2007 and 45 females and 36 males in 2008, of which 21 females and 12 males were measured in both seasons. Additionally, in 2008 ten females were captured twice during the season (36 ± 2 days difference between captures). We could obtain egg measurements of 28 clutches (9 in 2007, 19 in 2008). Normality was tested for each data set with Kolmogorov-Smirnov tests. If necessary (no normal distribution) we transformed variables prior to the analysis. We used natural logarithm transformation for numbers of leucocytes, lymphocytes and heterophils and plasma carotenoid concentrations. H/L ratios and chick age were normalized using a square-root transformation. For birds that were sampled more than once we used only the first measurement of each individual to avoid pseudoreplication. We tested for the effects of sex and annual variation on haematological and plasma biochemistry parameters using analysis of variance (ANOVA). Given our results (see below) we then standardized (mean = 0, SD = 1) numbers of leucocytes and lymphocytes in females separately for each year (see Hõrak et al., 2002; Ochs and Dawson, 2008).

To test whether haematological and plasma biochemistry parameters could be predicted from body condition, Julian date of capture, Julian date of hatching and chick age, we performed stepwise linear regressions, with probability of 0.05 for entry and 0.10 for removal (Ochs and Dawson, 2008) separately for males and females. For the analysis of the relationship between haematological and plasma biochemistry parameters and reproductive investment in females, we used clutch volume and mean egg volume as possible explanatory variables in stepwise regression models.

We then examined the variation in haematological and blood chemistry parameters within individuals sampled in both breeding seasons. Repeatabilities were calculated as intraclass correlation coefficients according to Lessells and Boag (1987). We tested whether the differences in haematological and blood chemistry parameters between two breeding seasons could be explained by differences in body condition, chick age, capture date and hatching date also using a series of stepwise multiple regressions (P to enter = 0.05, P to exit = 0.10). For the ten females recaptured within 2008 we tested whether the differences were related to differences in body condition and chick age.

For the comparison of haematological and plasma biochemistry parameters between pair partners we used Pearson correlations. Sample sizes differed because of missing values. Some territories were not visited before chicks hatched and hence data on clutch size and egg volumes are missing. Furthermore, not all haematological and blood biochemistry measures could be determined for all individuals in case of a low quality of blood smears or missing amount of plasma for all analyses. Statistical tests were performed in SPSS 11.0. Means are given with standard errors. Significance level was set to $P < 0.05$.

3. Results

3.1. Differences between sexes and years

Mean values of the haematological and plasma biochemistry parameters of adult Upland geese in the wild are shown in Table 1. We found lymphocytes the most abundant leucocytes of adult Upland geese (Table 1), which is in line with other studies in waterfowl (Artacho et al., 2007; e.g. Matson et al., 2006). We found no significant differences between males and females in H/L ratios, numbers of leucocytes, heterophils and lymphocytes per 10,000 erythrocytes and plasma protein and triglyceride concentrations (ANOVA, all $P > 0.1$). There was a significant difference in plasma carotenoids with females showing lower concentrations than males ($F_{1,117} = 17.884$, $P < 0.001$). There were no differences between the study years apart from the numbers of leucocytes and lymphocytes/10,000 erythrocytes in females, which were lower in 2007 than in 2008 (ANOVA, leucocytes: $F_{1,59} = 6.205$, $P = 0.016$, lymphocytes: $F_{1,59} = 7.568$, $P = 0.008$).

Table 1
Mean values of heterophils/lymphocytes ratios (H/L ratio), differential leucocyte counts and plasma biochemistry parameters of adult Upland geese in the wild. We tested for differences between the sexes and years using analysis of variance (ANOVA) of transformed values (see Statistical analysis section).

	Males			Females		
	Mean + SE	Range	*n*	Mean + SE	Range	*n*
H/L	0.44 ± 0.04	0.03–1.38	56	0.38 ± 0.03	0.02–0.96	60
Leucocytes/10,000Erythrocytes	35.59 ± 2.26	5.48–85.01	56	39.78 ± 2.51	9.55–83.98	60
Heterophiles/10,000Erythrocytes	10.13 ± 1.01	0.43–32.21	56	10.06 ± 0.89	0.71–34.43	60
Lymphocytes/10,000Erythrocytes	23.26 ± 1.41	4.18–58.89	56	27.07 ± 1.68	6.47–55.71	60
Carotenoids (µg/mL)	20.11 ± 0.68	12.71–33.76	57	16.27 ± 0.81	6.05–31.48	61
Plasma protein (g/L)	52.35 ± 1.28	31.98–87.83	58	51.76 ± 0.80	39.87–68.40	60
Plasma triglycerides (g/L)	1429.5 ± 51.7	608.2–2589.4	58	1529.7 ± 66.5	812.9–4341.8	62

3.2. Males

Stepwise multiple regression ($F_{2,56}=7.604$, $P=0.001$) suggested that H/L ratios increased significantly with the date of capture ($t=2.96$, $P=0.005$, Fig. 1b), and were higher in individuals with a lower body condition ($t=-2.60$, $P=0.012$, Fig. 1a). These relationships were driven by variation in the numbers of heterophils ($F_{2,56}=8.66$, $P=0.001$), that increased with decreasing body condition ($t=-2.96$, $P=0.005$, Fig. 1a) and later date of capture ($t=2.97$, $P=0.004$, Fig. 1b). Leucocyte and lymphocyte numbers and plasma biochemistry parameters could not be explained by any of the independent variables.

3.3. Females

Hatching date was the only variable in stepwise regressions explaining the variation in numbers of total leucocytes, heterophils and lymphocytes with all variables showing higher numbers in females with later hatching date (Fig. 2, leucocytes: $F_{1,58}=5.926$, $t=2.434$, $P=0.018$, heterophils: $F_{1,58}=5.661$, $t=2.379$, $P=0.021$, lymphocytes: $F_{1,58}=5.406$, $t=2.325$, $P=0.024$). Variation in chick age was significantly related to variation in plasma carotenoid concentration ($F_{1,58}=19.993$, $t=4.471$, $P<0.001$) with lower carotenoid concentrations in females with younger chicks. H/L ratios and plasma protein and triglyceride concentrations could not be explained by any of the independent variables in these multiple regression models.

Stepwise multiple regression also suggested that leucocyte counts decreased significantly with clutch volume (Fig. 3, $F_{1,27}=5.809$, $t=-2.410$, $P=0.023$), females that had laid larger clutches had lower numbers of circulating leucocytes. This relationship was driven by variability in numbers of lymphocytes which decreased with clutch volume (Fig. 3, $F_{1,27}=7.440$, $t=-2.728$, $P=0.011$). Also plasma protein concentration showed a trend towards higher levels in females with larger clutch volumes ($F_{1,27}=3.98$, $t=2.00$, $P=0.057$), whereas plasma carotenoid concentrations were higher in females that had laid eggs with a larger mean egg volume ($F_{1,27}=5.458$, $t=2.336$, $P=0.027$). H/L ratios, numbers of heterophils and plasma triglyceride concentrations could not be explained by variation in clutch and mean egg volume.

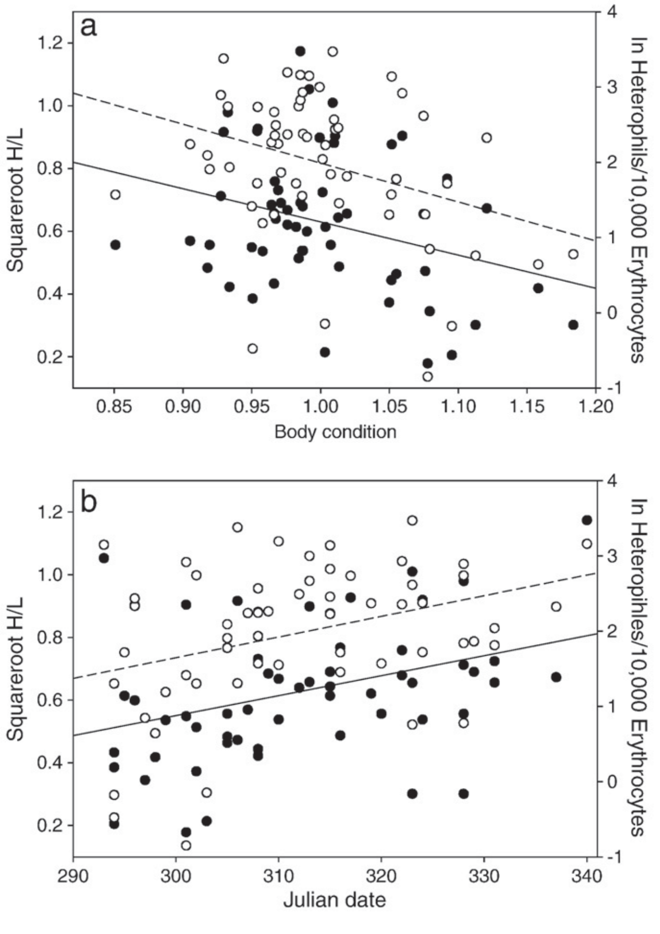

Fig. 1. Relationship between H/L ratio (black dots, solid line) and numbers of heterophils/10,000 erythrocytes (white dots, dashed line) and body condition (a) and capture date (b) of male breeding Upland geese on New Island. See text for Statistical analysis.

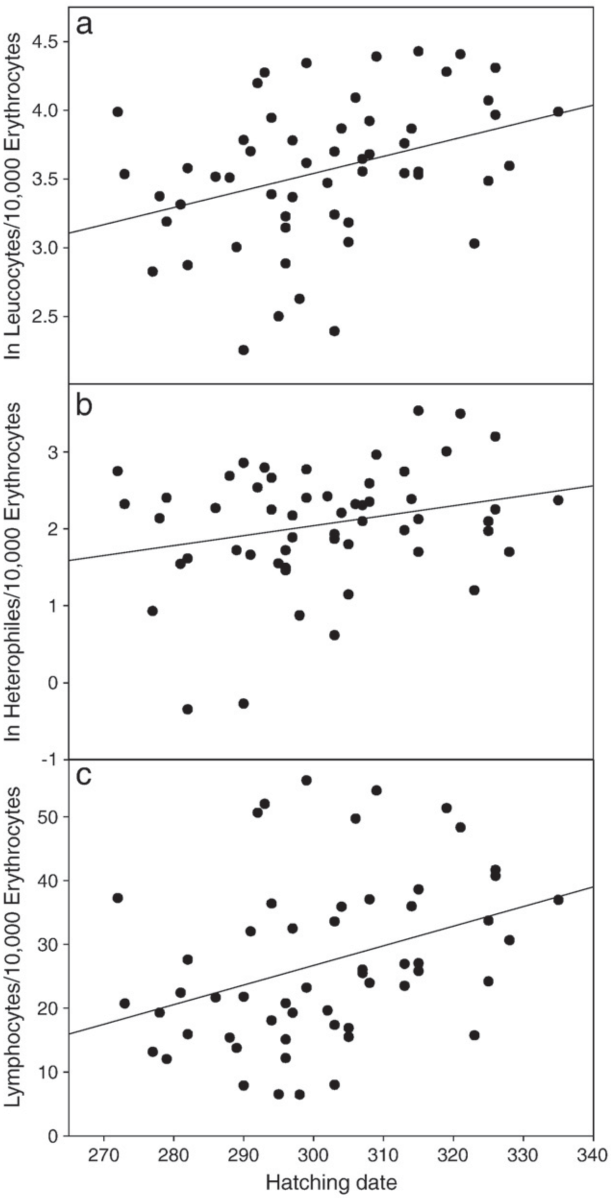

Fig. 2. Relationship between numbers of leucocytes (a), heterophils (b) and lymphocytes (c) per 10,000 erythrocytes and hatching date of female breeding Upland geese on New Island. See text for Statistical analysis.

3.4. Repeatability of haematological and plasma biochemistry parameters within individuals and influences on variability

In individual males, neither haematological nor plasma biochemistry parameters were significantly repeatable between 2007 and 2008 (all $P>0.1$). Changes of haematological and plasma biochemistry

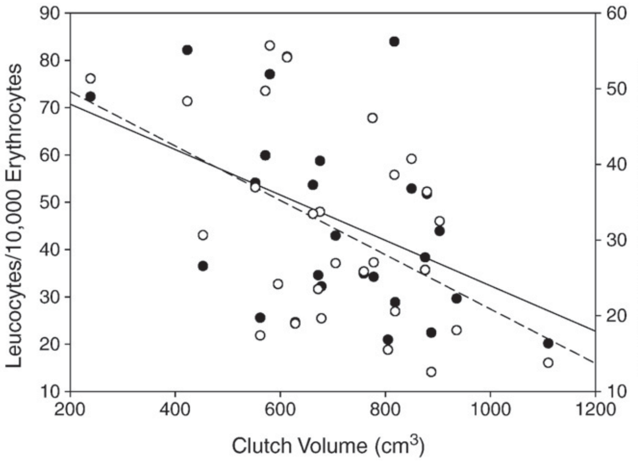

Fig. 3. Relationship between numbers of leucocytes/10,000 erythrocytes (black dots, solid line) and lymphocytes/10,000 erythrocytes (white dots, dashed line) and clutch volume of female breeding Upland geese on New Island. See text for Statistical analysis.

parameters were not influenced by changes in capture date, hatching date, chick age or body condition in multiple regression analyses (all $P>0.2$).

In individual females, only H/L ratio and plasma carotenoid concentrations showed a significant repeatability within individuals between 2007 and 2008 (H/L: $r=0.617$, $F_{18,38}=4.22$, $P=0.002$; carotenoids: $r=0.507$, $F_{18,38}=3.06$, $P=0.010$), counts of leucocytes, heterophils and lymphocytes per 10,000 erythrocytes and plasma protein and triglyceride concentrations were not significantly repeatable. Stepwise multiple regression showed that changes in leucocyte and lymphocyte counts were positively related to changes in capture date between the years (Fig. 4, leucocytes: $F_{1,17}=6.857$, $t=2.619$, $P=0.019$; lymphocytes: $F_{1,17}=7.878$, $t=2.807$, $P=0.013$). Changes in H/L ratios and heterophil counts were not influenced by changes in body condition, capture date, hatching date or chick age (all $P>0.1$). This analysis suggests that when the second sample was taken later in the year than the first sample, leucocytes and lymphocytes were more likely to be higher. Changes in plasma carotenoid concentrations were negatively related to changes in hatching date ($F_{1,17}=8.734$, $t=-2.955$ $P=0.009$), i.e. individuals with higher carotenoid concentrations in the second sample had hatched earlier. Changes in triglyceride and protein concentrations were not influenced by changes in any of the independent variables that we used in multiple regression analyses (all $P>0.2$).

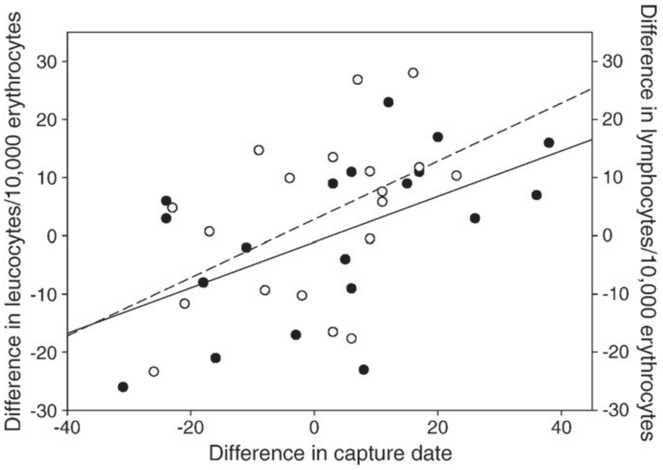

Fig. 4. Differences in numbers of leucocytes (black dots, solid line) and lymphocytes (white dots, dashed line) per 10,000 erythrocytes of female Upland geese sampled over two consecutive breeding seasons on New Island in relation to differences in capture date.

Within 2008, female individual H/L ratios, counts of heterophils per 10,000 erythrocytes and plasma protein concentrations were significantly repeatable (H/L: $r=0.658$, $F_{9,19}=4.85$, $P=0.011$, heterophils: $r=0.505$, $F_{9,19}=3.04$, $P=0.049$; plasma protein: $r=0.587$, $F_{8,17}=3.84$, $P=0.030$). We also rate plasma carotenoid concentrations as repeatable, although the p-value is slightly larger than 0.05 ($r=0.496$, $F_{9,19}=2.97$, $P=0.052$). Changes in haematological and plasma biochemistry parameters within 2008 were not significantly related to changes in chick age or body condition (all $P>0.2$).

3.5. Haematology and plasma biochemistry of pair partners

Numbers of leucocytes/10,000 erythrocytes, heterophils/10,000 erythrocytes and lymphocytes/10,000 erythrocytes of pair partners were significantly related (Pearson correlations; leucocytes: $r=0.45$, $N=57$, $P<0.001$, heterophils: $r=0.51$, $N=57$, $P<0.001$, lymphocytes: $r=0.39$, $N=57$, $P=0.002$, Fig. 5a–c). H/L was not significantly correlated in pair partners ($r=0.16$, $N=57$, $P=0.220$). Concentrations of plasma triglycerides of pair partners were significantly related, whereas plasma protein and carotenoid concentrations were not (Pearson correlations; triglycerides: $r=0.37$, $N=55$, $P=0.005$, proteins: $r=0.24$, $N=53$, $P=0.079$, carotenoids: $r=0.10$, $N=55$, $P=0.474$, Fig. 5d).

4. Discussion

4.1. Haematological and plasma biochemistry parameters and parental investment

Sex differences in haematological and plasma biochemistry parameters have been discussed to be caused by various reasons, like different endocrine profiles of males and females (Klein, 2000; Norte et al., 2009; e.g. Ots et al., 1998), differences in metabolism (Perez-Rodriguez et al., 2008a) or in parental investment (e.g. Hõrak et al., 1998a; Jakubas et al., 2008; Ots et al., 1998). We here report the case of no sex differences in haematological and most plasma biochemistry parameters, but a relation to different aspects of parental investment in male and female breeding Upland geese.

H/L ratios were related to body condition and capture date in males but not in females and this variation in H/L ratios was driven by differences in heterophil numbers. The stress related increase of H/L ratios has been shown by many studies and is a now well established tool in ecological research as this stress measure has the advantage of being less variable and longer lasting than the corticosterone stress response and multiple stressors can even have an additive effect (McFarlane and Curtis, 1989; McKee and Harrison, 1995). Higher H/L ratios in individuals in a low body condition and later in the year may reflect the investment of males into the establishment and defence of the breeding territory. Male Upland geese increase their aggressive activities gradually, besides evicting their own young from the previous breeding season, the territory has to be defended against intruding pairs looking for a breeding place and territorial boundaries with neighbouring pairs need to be maintained throughout the breeding season (Summers and McAdam, 1993). Aggressive encounters range from pacing side by side along the territorial boundaries over the exposure of their carpal knobs to gripping each other by the neck accompanied by the dashing of the wings against each other. All these activities are likely to be stressful to the birds, with low quality birds suffering more from fights than individuals in a good body condition and stress accumulating over the breeding season, which is indicated by the higher H/L ratios later in the year. Jakubas et al. (2008) also reported for Little auk (*Alle alle*) males to have increasing H/L ratios during the incubation period and attributed this to their additional activity in aggressive interactions in the colony.

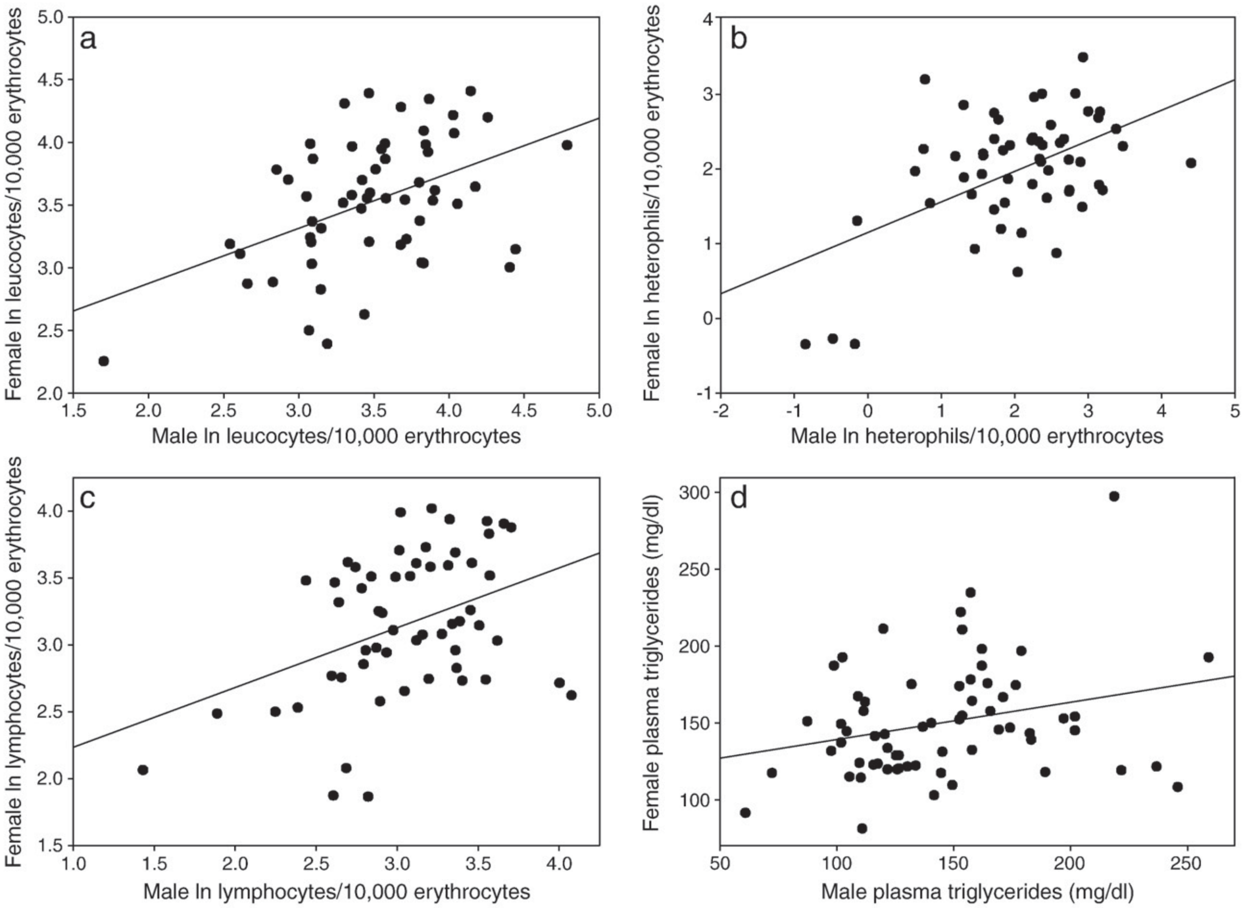

Fig. 5. Relationship of numbers of leucocytes (a), heterophils (b), lymphocytes (c) per 10,000 erythrocytes and (d) plasma triglyceride concentrations between pair partners of Upland geese on New Island. See text for Statistical analysis.

Only in female we found a relation of haematological and plasma biochemistry measures to reproductive parameters. Individuals with higher clutch volumes had lower total leucocyte numbers caused by lower numbers of lymphocytes. Poor health has been implicated as a factor which may affect reproductive performance (Gustafsson et al., 1994). As life-history theory suggests, individuals have to trade-off the investment in reproduction against the investment in body maintenance in any breeding season (Stearns, 1992). A higher exposure to infectious diseases as indicated by higher white blood cell counts and especially higher lymphocyte counts may decrease resources available for the investment into reproduction. The negative relationship we found between clutch volume and numbers of leucocytes and lymphocytes may indicate this cost of self maintenance. Low levels of circulating lymphocytes may indicate the absence of current infections requiring a specific immune response (Norris and Evans, 2000) or even low susceptibility to infections. Our results are in line with Moreno et al. (2002b) who reported that in Magellanic penguins (*Spheniscus magellanicus*) females with higher leucocyte counts layed smaller eggs and raised fewer chicks to fledging and Lobato et al. (2005) who found low leucocyte counts to be a good indicator of individual health state in Pied flycatchers (*Ficedula hypoleuca*). The lower numbers of all leucocyte types in females that hatched earlier also supports the interpretation of lower numbers as a sign for good condition and the allocation of resources into reproduction. Moreno et al. (1998) found in chinstrap penguins (*Pygoscelis antarctica*) late breeders to be in poorer health state indicated by high white blood cell counts. Early-nesting birds often lay bigger clutches; their offspring grow more rapidly and have a higher chance of survival and recruitment than late-nesting birds (Blums et al., 2002; Drent and Daan, 1980; Hochachka, 1990; Sockman et al., 2006), which results in a decrease in offspring value within a season. In the case of female Upland geese individuals fighting current infections might be allocating their resources to mount an immune response at the expense of the advantages of higher clutch size and early breeding. This is further supported by our finding that individual differences in lymphocyte numbers were related to changes in hatching date between the years, with females with higher numbers of lymphocytes in the second year hatching later. However, as haematological measures were taken after egg laying and incubation and due to the observational nature of our study it is difficult to establish causation. We therefore cannot exclude the possibility that increased reproductive effort in terms of high clutch volumes and early nesting lead to immunosuppression and therefore low lymphocyte counts (e.g. Hanssen et al., 2003; Hanssen et al., 2005; Sheldon and Verhulst, 1996). This dilemma in the interpretation of low lymphocyte counts as a sign for the lack of infections or alternatively immunosuppression has been pointed out before by Davis et al. (2008). Experimental studies including the sampling of birds before and after breeding and experiments measuring an individuals' response to an immune challenge are necessary to understand the relation of reproductive investment and haematological health parameters in this case. Also, experimentally increasing/reducing brood size could further highlight the importance of parental workload for leucocyte counts.

The analysis of plasma biochemistry also revealed that a good condition is reflected in reproductive investment in females. Plasma protein levels have been used as an indicator of body condition and protein availability for breeding in several studies (Dawson and Bortolotti, 1997; de le Court et al., 1995; Dunbar et al., 2005) and have been found to influence clutch size (Aboul-Ela et al., 1992; Beckerton and Middleton, 1982). In female Upland geese, lower levels of plasma protein were associated with lower clutch volumes and higher lymphocyte counts and may thus indicate that these females experience stress or diseases (Lewandowski et al., 1986). Also the higher values of plasma carotenoid concentrations in birds laying bigger eggs and earlier in the season indicate that females in a better state can invest their resources into reproduction. Egg size is an important component of parental effort in birds, larger eggs may enhance offspring fitness by increasing survival prospects in the first days after hatching and competitive power through a larger offspring size and the possession of more resources to survive adverse conditions (e.g. Amundsen and Stokland, 1990; Anderson and Alisauskas, 2002; Christians, 2002; Dawson and Clark, 2000; Erikstad et al., 1998).

4.2. Repeatability of haematological and plasma biochemistry parameters

Measures of individual quality should be relatively consistent over longer time spans to be a reliable indicator for the inherent quality of individual birds (Ochs and Dawson, 2008). Hõrak et al. (2002) suggested that if haematological parameters were relatively consistent within individuals, any among-individual differences might be a reflection of differences in levels of chronic stress. Alternatively, inconsistency of these measures within individuals may suggest that levels of stress vary over time (Vleck et al., 2000). In male Upland geese, leucocyte profiles and plasma biochemistry appear to be very variable between years as we found none of these parameters to be significantly repeatable between the two consecutive breeding seasons. Together with the result that H/L ratios are related to body condition and time of the season, we can conclude that leucocyte profiles in male Upland geese can be used as a measure of current stress but not as an indicator for the inherent quality of individuals. However, in females, the significant repeatability of H/L ratios both within and between seasons indicates that these measures might be used as an index for individual quality, which is in contrast with Ochs and Dawson (2008), who found H/L ratios not repeatable between two consecutive breeding seasons in female Tree swallows (*Tachycineta bicolor*) in the wild but similar to the results of Hõrak et al. (2002) who reports a significant repeatability of H/L ratios over a 4 months period in captive greenfinches (*Carduelis chloris*) with similar intraclass correlation coefficients. Despite this repeatability supporting the use of H/L ratios as measure of inherent quality, we found no relation to other quality measures like body condition. Monitoring over several years together with a complete data set for clutch measurements, hatching dates and body condition could shed further light on the usefulness of H/L ratios as a measure of individual quality in female Upland geese.

Also plasma carotenoid concentration showed repeatability only in female Upland geese. Together with the positive relation of carotenoid concentrations with egg size this supports the use of carotenoid concentrations as a quality measure. While reproductive parameters like hatching date or clutch size might be flexible and vary according to the current condition of a bird (as indicated by their relationship to levels of plasma protein and leucocyte counts), egg size may rather be related to inherent quality. Egg volume has been discussed in the context of individual quality before, with phenotypic and genetic factors predisposing some individuals to perform better under a given set of conditions and lay larger eggs (Ardia et al., 2006; Blackmer et al., 2005; Croxall et al., 1992; Reid and Boersma, 1990). Unfortunately, until now we do not possess enough data to reliably calculate individual repeatability of egg size in Upland geese, but egg sizes of two birds of which data in different seasons exist, were significantly repeatable ($r = 0.972$, $F_{1,3} = 70.78$, $P = 0.014$).

Furthermore, we found a significant relationship of plasma carotenoid concentrations with female tarsus colouration which itself was related to reproductive investment and repeatable between years (our unpublished data). Variability between individuals even when carotenoid access and health status are standardized indicates that intrinsic physiological or genetic factors may affect the ability to absorb, transport and transform carotenoids (Hadfield and Owens, 2006; McGraw and Hill, 2001). However, because Upland geese return to the same territories year after year, territory quality and carotenoid availability in territories may be an important factor in determining the between-year correlation in plasma carotenoid levels, and we cannot exclude this possibility without experiments.

4.3. Haematological and plasma biochemistry parameters of pair partners

The correlation of numbers of leucocytes, heterophils and lymphocytes in pair partners is a surprising result and to our knowledge, no study so far investigated haematological and plasma biochemistry parameters of pairs. There are several possible explanations for this pattern. One possibility is that the correlation of these haematological parameters is a sign for mate choice with individuals in a better immunological state being mated to individuals with the same characteristics. However, as none of these count variables showed a significant repeatability between the two years on an individual base, neither in males nor in females, this explanation is less likely. A more convincing conclusion may be that these measures reflect the current condition of an individual, which still may be similar in pair partners as we caught the male and female of a pair together at the same time, thus the recent conditions have been similar for both. Also the correlation of plasma triglyceride concentrations in pair partners may be explained by the fact that both partners are dwelling the same territory and facing the same small scale conditions. Plasma triglyceride concentrations are indicative of the nutritional state and especially changes in this state and thus closely linked to current environmental conditions. Sampling during the non-breeding period when Upland geese gather in large groups around ponds and thus share the same environmental conditions, could highlight the importance of the breeding territory or recent conditions.

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Can faecal glucocorticoid metabolites be used to monitor body condition in wild Upland geese *Chloephaga picta leucoptera*?

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Can faecal glucocorticoid metabolites be used to monitor body condition in wild Upland geese *Chloephaga picta leucoptera*?

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ABSTRACT

The measurement of faecal glucocorticoid metabolites (GCM) is used as a non-invasive technique to study stress in animal populations. They have been used most widely in mammals, and mammalian studies have also treated issues such as sample stability and storage methods. In birds, faecal corticosterone metabolite (CM) assays have been validated for a small number of species, and adequate storage under field conditions has not been addressed explicitly in previous studies. Furthermore, while it is well established that baseline plasma corticosterone levels in birds rise with declining body condition, no study so far investigated if this relationship can also be found using faecal samples. We here present data of a field study in wild Upland geese *Chloephaga picta leucoptera* on the Falkland Islands, testing different storage methods and investigating the relationship of faecal CM concentrations to body condition and reproductive parameters. We found that faecal CM measures are significantly repeatable within individuals, higher in individuals with lower body condition in both male and female wild Upland geese, and higher in later breeding females with smaller broods. These results suggest that measuring faecal CM values maybe a valuable non-invasive tool to monitor the relative condition or health of individuals and populations, especially in areas where there still is intense hunting practice.

Keywords: Upland goose, *Chloephaga picta leucoptera*, stress, body condition, faecal glucocorticoid metabolites

INTRODUCTION

The measurement of faecal glucocorticoid metabolites (GCM) in faeces has become a valuable tool in conservation biology and ecology to study stress load, enabling researchers to monitor the physiological state of wild animals both non-invasively and repeatedly. Faecal GCM have been used most widely in mammals, and mammalian studies have also treated issues such as sample stability and storage methods. One important question arising especially in studies in remote areas is the appropriate storage of faecal samples from the time of collection until later laboratory analysis. Studies with captive mammals showed that faecal samples are most stable when stored at sub-zero temperatures, followed by cool storage, drying or preservation in ethanol (reviewed in Wasser et al. 1988; Whitten et al. 1998; reviewed in Khan et al. 2002). However, as glucocorticoids are metabolised in a species-specific manner before excretion and assays need to be validated for each species, we wanted to test, whether these findings from studies in primates may be transferred to an avian system. A previous study by Koch et al. (2009) on captive Upland geese showed, that the concentrations of corticosterone metabolites (CM) of frozen and dried samples were highly correlated, indicating that the collection of droppings followed by drying might be an easy way to prepare samples for transport during fieldwork. However, Koch et al (2009) used a freeze-dryer, equipment that is not necessarily available under fieldwork conditions. We thus tested this method under fieldwork-condition by air-drying samples. Additionally we tested whether keeping droppings in ethanol directly after sampling gives comparable results to freezing. This could be especially important for humid regions, where air-drying might not be appropriate.

In our study we investigated whether CM values in Upland geese (*Chloephaga picta leucoptera*) are related to body condition and reproductive parameters like timing of breeding and number of offspring. Based on the Cort-Fitness Hypothesis (Bonier et al. 2009b) high glucocorticoid (i.e. corticosterone in birds) levels are often interpreted as a sign for individuals or populations in poor condition and linked to reduced fitness. These relationships have mainly been studied using baseline corticosterone concentrations in serum, but studies yield different results, varying within populations and even changing within individuals during their life history (reviewed in Bonier et al. 2009a). Faecal CM measures in birds have been linked to fitness in only a few studies (Kotrschal et al. 1998), although faeces have the advantage to provide a time-integrated measure of hormone levels compared to point measures in serum analyses (Millspaugh and Washburn 2004).

Upland geese belong to the order of the sheldgeese (Tadornini), a group that resembles true geese and shows similar habits but is more closely related to shelducks and ducks. The smaller (migratory) sub-species *C. picta picta* breeds on the South American mainland, whereas the slightly larger one (*C. picta leucoptera*) is restricted to the Falkland Islands. Their basic breeding biology and life-cycle has been studied in the Falklands from 1977 to 1980 (Summers 1983). At the New Island Nature Reserve, numbers of Upland geese have increased since 1973, when all livestock was removed from the island, to one of the highest population densities in the Falkland Islands (Quillfeldt et al. 2005). In other parts of their range though they are hunted intensely and there are few data available on their population and conservation status on both the South American mainland and the Falkland Islands. CM analysis in this case could be an easy, non-invasive method to study the effect of stressors like hunting, interactions with humans and habitat changes on important life-history parameters such as timing of breeding and number of offspring potentially affecting population development.

MATERIALS AND METHODS

Study site, field measurements and sampling

The study was carried out in the New Island Nature Reserve, Falkland Islands (51°43’S, 61°17’W) from October to December 2004, 2007 and 2008. In 2004, we observed unmarked birds (Quillfeldt et al. 2005), while the individually-based data of body condition, hatching date and brood size were obtained in 2007 and 2008. At the start of the 2007 and 2008 field seasons we mapped nests using GPS. For each nest, we determined clutch size, measured length (L, expressed in cm) and breadth (B, expressed in cm) of each egg to the nearest 0.1 mm using callipers and weighed each egg to the nearest 0.1 g using a digital balance. Egg volume (V; in cm^3) was calculated as $V = (L \times B^2 \times 0.507)$ following Furness and Furness (1981). We determined expected hatching dates as described in Gladbach et al. (2010a). Nests were visited at least once a day, starting at the estimated hatching date; all eggs hatched within 0-2 days from the estimated hatch date. In cases where nests were visiting after hatch of the chicks, hatching date was determined from a chick growth curve as described in Gladbach et al. (2010a).

We caught adults during the period when they attended their brood (mean chick age 12±2 days) using a 3 x 5 m whoosh net. One person herded the family of geese slowly to the catching area, and when they arrived directly in front of the furled net, the other researcher pulled the trigger. Adults were marked with individual metal rings and weighed to the nearest 10g using a digital spring balance. Head length, culmen length, and tarsus length were measured to the nearest 0.1 mm using callipers; wing length (maximum flattened chord) was measured to the nearest 1 mm using a stopped rule. We estimated a condition index correcting body mass for body size. This is considered to be a measure of nutrient reserves, where reserves are the quantity of utilizable tissues exceeding those required to meet daily nutritional demands. A Principal Components Analysis (PCA) extracted one principal component (PC1) from measurements of wing, head, bill and tarsus as an overall measure of body size in both males and females. In females, PC1 with an eigenvalue of 1.794 explained 44.844% of the variance. In males, PC1 with an eigenvalue of 2.001 explained 50.018% of the variance. The body condition for males and females was determined accounting for structural size, based on a regression of body mass on PC1. Body condition is expressed as the ratio of the observed body mass to the derived expected body mass, according to individual size.

Collection of faecal samples

Faecal samples were collected when families were leading broods (chick age at first sample: 32±2 days). Sampling took place between 10am and 12am, starting at a mean of 20±1 days after capture of adult birds. Families were approached until a dropping of both male and female was observed. If this took longer than ten minutes, the attempt was aborted. Droppings were collected and kept in plastic tubes on ice–packs in an insulated bag during a sampling session. After returning to the field station (usually within one hour), droppings were processed immediately. A sample was placed in a petri dish and mixed using a spatula. In 2004, one part was frozen at -20°C and the other part in a sunny window (usually dry within 1 day). In 2007, one part was mixed with ethanol (70%) in a new tube and the other part air-dried. In 2008, one part of the sample was mixed with ethanol in a new tube and of 47 samples, a further part was frozen at -20°C. Tubes of ethanol samples were sealed with a tap and parafilm to prevent leakage. In 2004, we collected a total of 33 samples from 16 males and 16 females. In 2007 and 2008 individuals were sampled repeatedly (3-6 times) with an interval of 3 to 5 days between sampling sessions. In 2007, we took a total of 125 samples from 15 males and 14 females. In 2008, we took a total of 132 samples from 13 males and 13 females, of which 47 were partly frozen.

Extraction and measurement of the excreted CM

In the lab, a subsample of 0.25 g of each fresh faecal sample was mixed with 0.75 ml double-distilled water followed by 1.5 ml methanol and vortexed for 30 min. After centrifugation (2500g, 10 min), the supernatant was transferred to a new tube and used for the analyses with the EIA described below.

A subsample of 0.08 g of dried samples was mixed with 1 ml double-distilled water, to compensate for the water loss during drying (see Koch et al. 2009), and 1.5 ml methanol before vortexing for 30 min. Ethanol samples were dried using a STERIS Lyovac GT- 2E freeze dryer and than treated like dried samples (see above).

Koch et al. (2009) tested various enzyme immunoassays (EIA) for CM in Upland geese and found an 11-oxoetiocholanolone-EIA (Möstl et al. 2002) measuring the highest peak values of CM in faeces of Upland geese after ACTH injection and this assay was more sensitive than other tested EIAs. After extraction, 20 μl aliquots were transferred into microtiter plates and measured using this assay. Intra-assay variation was 10.9%, inter-assay variation for the low-level pool was 15.6% and 13.2% for the high-level pool. All concentrations are given in nanogram per gram wet weight, assuming a constant water content. Because the actual water content may differ between samples, the final concentration between dry, ethanol and wet samples cannot be compared directly, but a correlation between the corresponding dry, ethanol samples and wet samples would indicate that the methods yield comparative results.

Statistical analysis

Statistical tests were performed in SPSS 11.0. Normality was tested with Kolmogorov-Smirnov tests. Means are given with standard errors. We assessed significance using General Linear Models (GLM). As a measure of effect sizes we included partial Eta-Squared-Values (η^2) in the tables (i.e. the proportion of the effect + error variance that is attributable to the effect). The sums of the partial Eta-squared-values are not additive (http://web.uccs.edu/lbecker/SPSS/glm_effectsize.htm). Significance level was set to p<0.05. Because CM values for the analysis of sample storage methods were not normally distributed we used a ln-transformation. For the analysis of chick age, body condition, reproductive parameters and CM values we used data from 2007 and 2008 only, while in 2004 birds were not captured. We analysed values of samples stored in ethanol, as this method was used in both 2007 and 2008. To ensure that CM data collected for each year were directly comparable in subsequent analyses, we standardized values of ethanol samples for each year separately (mean=0, SD=1) (see Hõrak et al. 2002; Ochs and Dawson 2008).

For the analysis of the influence of chick age on faecal CM concentrations we used a GLM for sexes separately with standardised CM values as dependent, individual identity as random factor and chick age as covariate. For the individuals captured in both years we used CM concentrations from the first year in the overall model to avoid pseudoreplication. To test, whether the same trend can be observed in both years we then ran an additional analysis for each year separately, including all captured individuals (Tab.1). As CM concentrations might be related to changes in stressors occurring with increasing chick age, we additionally tested whether CM were related to age of the chick when the first sample was taken. We ran a GLM with CM as dependent, sex as fixed factor and body condition and chick age at first sample as covariates. We also included the interaction between sex*chick age.

For the analysis of body condition and reproductive parameters we used a mean value for all individuals. We here also included the measurement from the first year if individuals were captured in both 2007 and 2008. We ran GLMs with CM values as dependent, sex as factor, body condition as covariate and the interaction of sex and body condition). As females lose weight during incubation which they regain after hatching of the chicks and there was a time lag in our study between the capture of animals and faecal sampling, we ran a second analysis for females: In 2008 we recaptured 10 females with a mean of 35 days after the first capture. We found a mean daily mass increase of 3 gram. Using this information, we calculated a hypothetical body condition for each female (adding a hypothetical mass to the measured mass by multiplying the days between capture and first hormone sample with 3g). This resulted in a mean increase in body condition of 0.02 points. We ran the tests again using this hypothetical body condition for females and the results remained qualitatively the same. As we sampled pair partners, we analysed the relation of CM values and hatching date or brood size for males and females separately using Pearson correlation to avoid pseudoreplication of hatching date and brood size.

RESULTS

Comparison of sample storage methods

CM values of frozen and air-dried samples were significantly correlated (Pearson correlation, $R = 0.740$, $P < 0.001$, $N = 31$, Fig.1a). CM contents of ethanol samples and frozen samples were significantly correlated ($R = 0.506$, $P < 0.001$, $N = 47$, Fig.1b), as well as CM measured in ethanol samples and dried samples ($R = 0.691$, $P < 0.001$, $N = 125$, Fig.1c). In 2008 we analysed 18 ethanol samples twice (subsamples were created at the weighing stage). The correlation between both subsamples was highly significant ($r=0.85$, $P<0.001$), which indicates that storage in ethanol gives reliable results.

Influence of chick age within individuals

The overall model showed that faecal CM concentrations decreased with increasing chick age in both males and females and there were significant individual differences (Tab.1). This trend could be found in both 2007 and 2008 (Tab.1). The effect size for inter-individual differences in CM concentrations was higher than for intra-individual differences with increasing chick age (Tab.1). Chick age at first sample had no effect on CM concentrations (GLM, factor sex: $F_{1,45} = 1.182$, $P = 0.185$, body condition: $F_{1,45} = 5.905$, $P = 0.020$, chick age: $F_{1,45} = 2.388$, $P = 0.130$, interaction sex*chick age: $F_{1,45} = 2.469$, $P = 0.124$).

We found faecal CM concentrations within individuals to be significantly repeatable for both males and females (males: $r = 0.332$, $P < 0.001$, N=28; females: $r = 0.158$, $P = 0.005$, N=29).

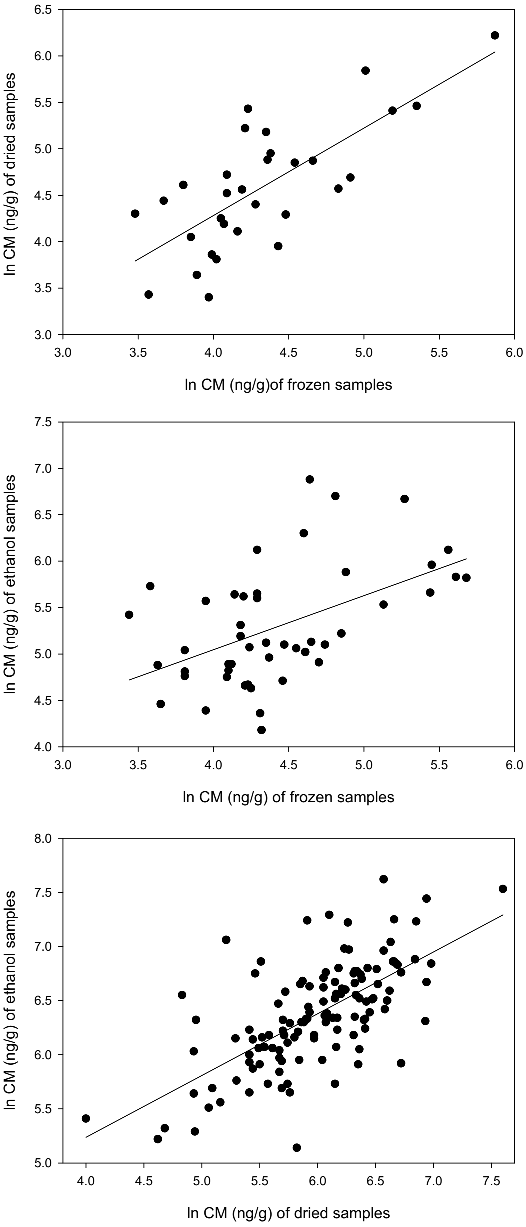

Fig. 1 Pairwise comparisons of different storage methods on corticosterone metabolite (CM) concentrations in faecal samples of Upland geese on New Island. (a) frozen – air-dried, samples from 2004; (b) frozen – ethanol, samples from 2008; (c) air-dried – ethanol, samples from 2007.

Influence of sex, body condition and reproductive parameters

There was no significant difference in CM values between males and females ($F_{1,45} = 0.600$, $P = 0.443$) during the time when they were leading chicks. We found a tendency of CM values to be related in pair partners (Partial correlation, controlling for year: $R = 0.348$, $P = 0.089$, df = 23). CM values were significantly negatively related to body condition in both males and females with individuals in better condition showing lower values (GLM, factor sex: $F_{1,45} = 0.002$, $P = 0.966$,

$\eta^2 < 0.001$, body condition: $F_{1,45} = 10.993$, $P = 0.002$, $\eta^2 = 0.211$, $t = -2.467$, interaction sex*body condition: $F_{1,45} = 0.004$, $P = 0.948$, $\eta^2 < 0.001$, Fig. 2a). In females, these results remained qualitatively the same using the hypothetical body condition (GLM, hypoth. body condition: $F_{1,22}= 12.257$, $P = 0.002$, $\eta^2 = 0.280$, $t = -3.501$)

Hatching date was positively related to CM values in females, with individuals with lower CM values starting to reproduce earlier in a season (Pearson correlation: $R = 0.686$, $P < 0.001$, $N = 22$; Figure 2b). CM values were not related to hatching date in males ($R = -0.199$, $P = 0.362$, $N = 23$; Fig. 2b).

The brood size (number of chicks per brood) was negatively related to CM values in females ($R = -0.543$, $P = 0.009$, $N = 22$; Figure 2c) but not in males ($R = -0.374$, $P = 0.079$, $N = 23$; Fig. 2c).

DISCUSSION

Comparison of sampling methods

We found a significant correlation between all pair wise compared sampling storage methods. The highest correlation coefficient was observed between frozen and air-dried samples. The correlation coefficient is lower than the one reported by Koch et al. (2009), who dried samples directly in the laboratory using a freeze dryer, but the highly significant result in our case still supports the use of air-drying under fieldwork-conditions. Oven-drying of faecal samples in field sites without electricity or organic solvents has been used for instance in primate studies (Brockman and Whitten 1996; Whitten et al. 1998) and proved to be a useful preservation technique there as well. However, caution is necessary here as well as also drying temperature may affect hormone levels (e.g. Terio et al. 2002).

The correlation between ethanol and frozen samples was also highly significant in our study. However, the correlation coefficient was lower than for air-dried samples, indicating that storage in ethanol might increase variation in CM measures more than air-drying. The way the storage of faecal samples in ethanol affects hormone concentrations is still discussed in the literature. For example, Whitten et al. (1998) found that the majority of steroids are extracted into the solvent when faeces are kept for prolonged periods in 90% ethanol at ambient temperatures and Khan et al. (2002) suggest that immunoreactive metabolites might have a higher extraction efficiency when stored in ethanol for several months at ambient temperature, caused by the deposition of metabolites on the surface of the faecal material during ethanol evaporation and freeze-drying, which then go more easily into solution during extraction. Based on our empirical results, we would therefore recommend the use of air-drying if possible. Storage in ethanol is also valid, but less preferable. Dried samples are easier to transport as they take less space and do not need to be sealed to avoid leakage of alcohol.

Repeatability and influence of chick age within individuals

Studies in other goose species suggest, that the faeces contain an integrated, proportional record of the plasma corticosterone levels approximately 1h before defaecation (Kotrschal et al. 1998; Hirschenhauser et al. 2000; Mostl et al. 2005), however metabolites are excreted faster in urine (after 1-2 hours) than in the faeces (3 hours) (Mostl et al. 2005). In birds, the cloaca serves for the excretion of both faeces and urine (uric acid) and in some species, urine in droppings can be sampled separately from the faeces (Wasser et al. 1997; Klasing 2005). In Upland geese the urinary part is hard to distinguish from the faecal part (personal observation). The study by Koch et al. (2009) showed a peak 2-3h after the injection of an ACTH

Tab 1 Effect of individual identity and chick age on faecal CM concentrations of wild Upland geese on New Island. GLMs with standardised CM values as dependent, individual identity as random factor and chick age as covariate. Partial Eta-Squared-Values (η^2) denote the effect size and t-values the direction of the effect.

		Ring	chick age
females	overall	$F_{21,71} = 1.666$, $P = 0.058$, $\eta^2 = 0.330$	$F_{1,71} = 9.391$, **$P < 0.001$**, $\eta^2 = 0.169$, $t = -3.803$
	2007	$F_{13,44} = 1.256$, $P = 0.276$, $\eta^2 = 0.271$	$F_{1,44} = 9.391$, $P =$ **0.004**, $\eta^2 = 0.176$, $t = -3.064$
	2008	$F_{12,47} = 2.207$, **$P = 0.027$**, $\eta^2 = 0.361$	$F_{1,47} = 4.056$, $P =$ **0.049**, $\eta^2 = 0.079$, $t = -2.014$
males	overall	$F_{21,76} = 3.706$, **$P < 0.001$**, $\eta^2 = 0.506$	$F_{1,76} = 11.323$, $P =$ **0.001**, $\eta^2 = 0.130$, $t = -3.365$
	2007	$F_{13,46} = 3.495$, **$P = 0.001$**, $\eta^2 = 0.497$	$F_{1,46} = 9.300$, $P =$ **0.004**, $\eta^2 = 0.168$, $t = -3.050$
	2008	$F_{11,45} = 3.910$, **$P = 0.001$**, $\eta^2 = 0.489$	$F_{1,45} = 3.319$, $P = 0.075$, $\eta^2 = 0.069$, $t = -1.822$

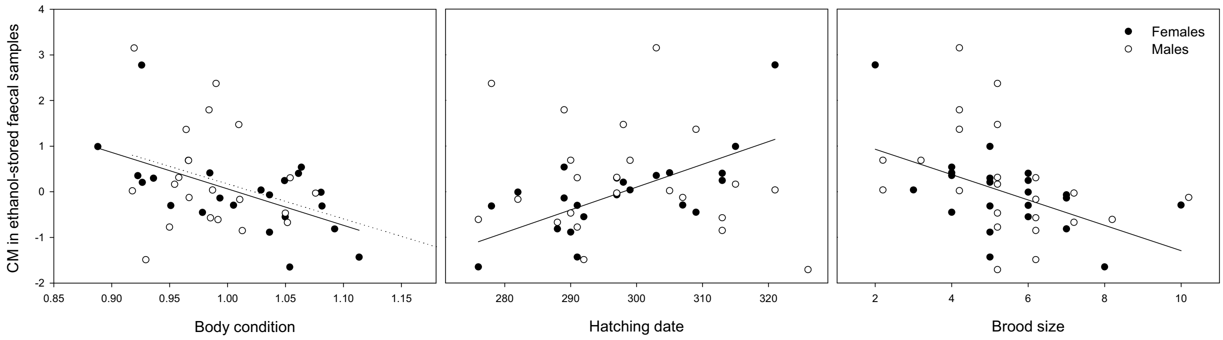

Fig 2 Relationship between (a) body condition, (b) hatching date and (c) brood size and corticosterone metabolite (CM) values in faecal samples of female (black dots, solid line) and male (white dots, dotted line) Upland geese on New Island. CM values were standardized for each year separately to ensure that data collected were directly comparable. Regression line only for significant relationships. See text for statistical analysis

analogue, indicating that metabolites mainly excreted in the faeces were measured by the assay. The time span is especially important to determine, when a specific stressful event took place. However, in our study we were interested in baseline levels. In both males and females, faecal CM concentrations from consecutive samples were highly repeatable; indicating that we measured baseline levels and that there is considerable individual variation. This is further supported by the higher effect size of individual identity in our model testing the influence of chick age and identity. Variation in baseline corticosterone levels has been found to be repeatable in geese before (Kralj-Fiser et al. 2007) and partly heritable in several other avian species (Satterlee and Johnson 1988; Evans et al. 2006), thus possibly providing an honest signal of individual quality with lower quality individuals perceiving their environment as more challenging (Bonier et al. 2009a).

We found that in both males and females, faecal CM concentrations decreased with increasing chick age. We propose three possible and not mutually exclusive explanations for this pattern:

1) With increasing chick age, chicks become more independent and have a lower risk to be taken by the main predators, Striated caracaras (*Phalcoboenus australis*) and Falkland Skua (*Catharacta antarctica*). They are also less vulnerable to be killed during territorial fights by neighbouring ganders, which regularly kill goslings by picking or even throwing them (personal observations). Parents can thus decrease their vigilance and concentrate more on foraging and self maintenance.

2) Both males and females regenerate their body condition after the high investment at the beginning of the breeding season. Females are known to lose weight during incubation which is regained after hatching (Summers and McAdam 1993). Males on the other hand invest heavily into the establishment and defence of the breeding territory. With advancing season, these fights become less violent as territory boundaries are established and only few geese (presumably first breeders) try to establish a new territory and start breeding (personal observation). In both males and females this could affect individual stress load and condition.

3) Environmental conditions change with advancing season. While October and November are usually relatively dry, precipitation starts to increase in December, improving the freshwater supply, that Upland geese depend upon and the amount of vegetation available for foraging. These more favourable conditions may relax individual perceived stress levels.

Relation to body condition

We found that in both males and females, faecal CM concentrations were negatively related to body condition, with individuals in better condition showing lower values. As pointed out by Husak and Moore (2008), stress hormone levels have been linked to body condition in a variety of animals (Moore and Jessop 2003) based on the role of glucocorticoid hormones in energy mobilisation, although cause and effect remain unclear (Husak and Moore 2008). Studies in avian systems often measure baseline corticosterone levels in plasma samples and report a condition dependence of corticosterone levels (Perez-Rodriguez et al. 2006). To our knowledge, no study so far investigated if this relationship can also be found using faecal samples, as normally faecal samples are specifically used as a non-invasive technique without capturing focal animals. The use of faecal samples might be advantageous when studying the relationship between corticosterone and individual condition, because they provide a time-integrated measure of hormone levels compared to point measures in blood plasma analyses. Individuals showed a clear correlation of CM values and body condition despite the time lag of 20 days between the two measurements. Our data thus suggest that Upland Geese in good body condition conserved their good condition throughout the chick-rearing season.

Relation to reproductive parameters

We found that only in female Upland geese CM values were related to hatching date and brood size, with higher CM values in later breeding individuals and in individuals with smaller broods. A recent study by Schoech et al. (2009) found a similar pattern in Florida scrub-jays *Aphelocoma coerulescens*, where corticosterone levels were also positively associated with timing of reproduction in females. In Upland geese, males and females differ in their specific parental roles, with males establishing and intensely defending the territory and females incubating and brooding (Summers and McAdam 1993; Gladbach et al. 2010b). Our present findings thus fit well into this pattern, as number of offspring and timing of breeding depend mainly on female resources and condition (Gladbach et al. 2010a). Early-nesting birds often lay bigger clutches; their offspring grow more rapidly and have a higher chance of survival and recruitment than late-nesting birds (Drent and Daan 1980; Hochachka 1990; Blums et al. 2002; Sockman et al. 2006), which results in a decrease in offspring value within a season. The earlier hatching dates and larger brood sizes of females with lower CM values may be explained by differences in individual quality. Low quality individuals might secrete corticosteroids at higher levels (Wingfield and Sapolsky 2003; Husak and Moore 2008; Bonier et al. 2009a) either because they perceive certain environmental circumstances as more challenging per se or because these circumstances are more challenging to them due to their lower condition. As elevated corticosterone levels are assumed to reallocate resources away from reproduction (Bonier et al. 2009a), onset of incubation (and thereby hatching date) might be postponed in low quality individuals. Brood size could then be smaller either because the expected offspring value is assumed to decline during a breeding season or because lower quality individuals are not able to invest as heavily into reproduction as high quality birds. The observational nature of this study cannot clarify causality, but our empirical results can be a good basis to be used in conservation practice.

Summary

We found a significant correlation between all pair wise compared sampling storage methods, with air-dried samples being best correlated to frozen samples followed by ethanol-stored samples. Faecal CM measures are significantly repeatable within individuals, higher in individuals with lower body condition in both male and female wild Upland geese, and higher in later breeding females with smaller broods. The results were strong despite the fact, that ethanol storage might not have been optimal compared to drying. Our results suggest that measuring faecal CM values may be a valuable non-invasive tool to monitor the relative condition or health of individuals and populations, especially in areas where there still is intense hunting practice.

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Female-specific colouration, carotenoids and reproductive investment in a dichromatic species, the Upland goose *Chloephaga picta leucoptera*

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ORIGINAL PAPER

Female-specific colouration, carotenoids and reproductive investment in a dichromatic species, the upland goose *Chloephaga picta leucoptera*

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Abstract Although studies on the evolution and function of female ornaments have become more numerous in the last years, the majority of these studies were carried out in cases where female ornaments were a smaller and duller version of the ornaments found in males. There are substantially fewer studies on species with female-specific ornaments. However, no study so far investigated the potential of female-specific colouration as a quality signal in birds with conventional sex roles. We studied female-specific ornamentation in a strongly sexually dichromatic species, the upland goose *Chloephaga picta leucoptera*, in two consecutive years. Male upland geese have white head and breast feathers and black legs, whereas females have reddish-brown head and breast feathers and conspicuous yellow-orange legs. We found that female-specific colouration in upland geese can reliably indicate different aspects of female phenotypic quality. Females with more orange coloured legs and more red-like head colours had higher clutch and egg volumes than females with a paler leg and head colouration, and a more reddish plumage colouration was related to a higher body condition. These relationships provide the theoretic possibility for males to assess female phenotypic quality on the basis of colouration. Furthermore, the females with a more orange-like tarsus colouration had higher plasma carotenoid levels. Both tarsus colouration and carotenoid concentrations of individual females were highly correlated across years, indicating that tarsus colour is a stable signal. Despite this correlation, small individual differences in plasma carotenoid concentrations between the two study years were related to differences in tarsus colouration. We thus show for the first time in a wild bird and under natural conditions that carotenoid-based integument colouration remains consistent between individuals in consecutive years and is also a dynamic trait reflecting individual changes in carotenoid levels. In this species, where pairs form life-long bonds, the honesty of the carotenoid-based integument colouration suggests that it may be a sexually selected female ornament that has evolved through male mate choice.

Keywords Upland goose · *Chloephaga picta leucoptera* · Female-specific colouration · Signalling · Carotenoids · Individual quality

Introduction

Female ornamentation has long been interpreted as a by-product of a genetic correlation to a selected trait in males that has no function in females (Lande 1980). After studies could demonstrate that female ornaments can have a

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signalling function (Jones and Hunter 1993; Potti and Merino 1996; Amundsen et al. 1997; Amundsen and Forsgren 2001), it is now acknowledged that natural and/or sexual selection also acts directly on ornaments in females and can explain their development (Amundsen 2000; Clutton-Brock 2009). Selection processes for ornamentation in both males and females are mate choice and mate competition, competition over resources other than mates and selection for sexual mimicry (Kraaijeveld et al. 2007). Most studies on the signalling function of female ornaments have been carried out in species where the female ornament is a reduced form of a sexually selected trait in males, and ornamentation could be linked to female quality and fecundity, although results are mixed with some studies reporting no or negative relationships (reviewed in Amundsen 2000; Kraaijeveld et al. 2007; Clutton-Brock 2009).

There are substantially fewer studies on species with female-specific ornaments. In a number of non-avian species where females display ornaments that are not present in males, it has been demonstrated that these female-specific ornaments can have signalling function as well (Funk and Tallamy 2000; Amundsen and Forsgren 2001; Domb and Pagel 2001; Weiss 2006; Weiss et al. 2009). Besides the amount of literature on female ornaments in birds compared to other taxa, also in birds, most of the empirical work on female ornamentation has been carried out in species with similar ornaments in males and females (Amundsen 2000; Bennett and Owens 2002), maybe because the situation where both males and females are ornamented but have different ornaments appears to be rare. Heinsohn et al. (2005), for example, explored the reasons for a strikingly different plumage colouration in the *Eclectus roratus* parrot and found different selection pressures acting on males and females, attributed to sex-based differences in their lifestyle. However, their study does not link any of the plumage characters to measures of individual quality, and to our knowledge, no study so far investigated the potential of female-specific colouration as a quality signal in birds with conventional sex roles.

We studied female-specific colouration in a strongly sexually dimorphic species, the upland goose *Chloephaga picta leucoptera*. Upland geese are highly territorial and socially monogamous, usually returning to the same territory with the same mate every year (Summers and McAdam 1993). They belong to the order of the sheldgeese (Tadornini), a group that resembles true geese and shows similar habits but is more closely related to shelducks and ducks. Male upland geese have white head and breast feathers and black legs, whereas females have reddish-brown head and breast feathers and conspicuous yellow-orange legs (Fig. S1). Several studies on a variety of species have shown evidence that integument colouration (e.g. bill, skin) reliably signals individual quality or state (e.g. Negro et al. 1998; Faivre et al. 2003; Velando et al. 2005; e.g. Velando et al. 2006; Martinez-Padilla et al. 2007; Mougeot et al. 2007b, 2009). In contrast to plumage colouration, which may mostly reflect the conditions during moult and not necessarily condition at the time the study is conducted, the colour of skin or other integumentary parts reflects a more recent physiological state and hence is a more plastic indicator of current condition (Lozano 1994; Negro et al. 1998; Bortolotti et al. 2003; Martinez-Padilla et al. 2007). However, integument colouration may change rapidly (Faivre et al. 2003; Rosen and Tarvin 2006; Velando et al. 2006), and reliability can only be sustained if these changes reflect current state or the change of the state from one year to the next and if the relative state of an individual compared to others is stable (Greenfield and Rodriguez 2004; Senar and Quesada 2006; Perez-Rodriguez 2008). As Perez-Rodriguez (2008) could show for captive red-legged partridge *Alectoris rufa*, carotenoid-based integument colouration can be consistent between individuals both within and between years.

In this study, we examined whether the expression of female-specific colouration can reliably predict female phenotypic quality in the upland goose. Our study will thus be the first to test if female-specific colouration varies in expression and that this variation may have some signal value in a bird species with conventional sex roles. Furthermore, no study so far investigated the stability of integument colouration in consecutive years for wild birds under natural conditions. Our specific aims were as follows. We tested:

1. Whether female colouration was positively related to her reproductive investment, in terms of clutch and egg size. Egg size is an important component of parental effort in birds and several studies have shown a positive relationship between egg size and offspring fitness, in altricial as well as precocial birds. Larger eggs may enhance offspring fitness by increasing survival prospects in the first days after hatching and competitive power through a larger offspring size and the possession of more resources to survive adverse conditions (Ankney 1980; Amundsen and Stokland 1990; Magrath 1992; Potti 1999; Dawson and Clark 2000; Anderson and Alisauskas 2002; Christians 2002; Goth and Evans 2004; Rutkowska and Cichon 2005). In our system, we found that chicks hatched from nests with higher mean egg volume had higher early body condition (Gladbach et al. 2010).
2. Whether female colour was repeatable across years, an important factor in the reliability of a variable signal like integument colouration, which has never been investigated in the wild. Measures of individual quality need to be relatively consistent and repeatable over time in order to reflect the state of an individual over

long time spans and reveal information about the inherent quality of individuals. To test this in the upland goose, we sampled individuals over two consecutive breeding seasons.

3. Whether variation in tarsus colour correlates with variation in plasma carotenoid levels both among and within individuals, indicating if changes in integument colouration may reflect individual differences and changes in carotenoid levels. In many species, the yellow, orange or red colour of integumentary parts results from the presence of carotenoids (McGraw 2004; Mougeot et al. 2007a; Juola et al. 2008; Perez-Rodriguez 2008). Carotenoids are natural pigments that cannot be synthesised by vertebrates and hence must be obtained via the diet (Fox 1979; Brush 1981; McGraw 2005). Therefore, the expression of carotenoid-based ornaments can indicate good foraging efficiency and condition (Endler 1983; Hill et al. 1994; Moller et al. 2000) or a high carotenoid availability in good territories. Carotenoids also have a range of health-related functions: they are known to work as antioxidants and immune enhancers (Lozano 1994; Olson and Owens 1998). This provides the basis for the honesty of carotenoid-derived signals because individuals may have to trade off the allocation of carotenoids to self-maintenance against ornamental colouration (Lozano 1994; von Schantz et al. 1996; Blas et al. 2006).

Materials and methods

Study site and study species

The study was carried out in the New Island Nature Reserve, Falkland Islands (51°43′S, 61°17′W) from October to December 2007 and 2008. The island has been established as a nature reserve in 1970 when all livestock was removed from the island. This led to an increase in the density of upland geese, which is now one of the highest in the Falkland Islands (Quillfeldt et al. 2005). The basic breeding biology and life cycle of upland geese has been studied in the Falklands from 1977 to 1980 (Summers 1983a). Take up of territories starts in August, egg laying commences in late September and most clutches are finished by the end of October. Hatching mainly takes place between mid-October and mid-November, and fledging starts when offspring are about 70 days old.

Field measurements and sampling

At the start of each field season, we mapped nests using Global Positioning System. For each nest, we determined clutch size, measured length (L, expressed in centimetres) and breadth (B, expressed in centimetres) of each egg to the nearest 0.1 mm using callipers and weighed each egg to the nearest 0.1 g using a digital balance. Egg volume (V, in cubic centimetres) was calculated as $V=(L\times B^2\times 0.507)$ following Furness and Furness (1981). We defined total clutch volume as the sum of the volumes of each egg in the clutch. We estimated expected hatching dates as described in Gladbach et al. (2010). We visited nests at least once a day, starting at the estimated hatching date; all eggs hatched within 0–2 days from the estimated hatch date.

We caught adults during the period when they attended their brood using a 3×5 m whoosh net. One person herded the family of geese slowly to the catching area, and when they arrived directly in front of the furled net, the other researcher pulled the trigger. Adults were marked with individual metal rings and weighed to the nearest 10 g using a digital spring balance. Head length, culmen length and tarsus length were measured to the nearest 0.1 mm using callipers; wing length (maximum flattened chord) was measured to the nearest 1 mm using a foot rule. A blood sample (approximately 300 μl) was collected from the brachial vein. Blood samples were kept cold (4°C) and centrifuged within 8 h. The separated plasma was stored at −20°C until carotenoid levels were determined (see below).

Chicks were weighed to the nearest 1 g using a spring balance (<300 g) or to the nearest 10 g using a digital spring balance (> 300 g). We measured head length, culmen length, wing length and tarsus length (±0.1 mm) using callipers to determine chick age from a growth curve for head and tarsus established from chicks of known age in 2005. For each clutch, the mean chick age and thereby mean hatching date were calculated. As not all territories were visited before hatching and thus hatching dates could not be determined from egg density for all individual females, we used this estimated hatching date for further analyses. Estimated hatching dates from egg measures and chick measures were highly correlated ($r=0.973$, $P<0.001$, $N=41$).

The body condition of females was determined accounting for structural size and chick age, as we did not catch females during the prelaying period and captured females had goslings of different age (mean age, 11±1 days) . Females lose weight during incubation and regain weight after hatching of the chicks (Summers and McAdam 1993). A principal components analysis extracted one principal component (PC1) with an eigenvalue of 1.794 from measurements of wing, head, bill and tarsus as an overall measure of body size. PC1 explained 44.844% of the variance. We estimated an expected body mass for each individual based on a multiple linear regression of body mass on the first principal component score (PC1) and chick age. Body condition was calculated as the ratio of the observed body mass to the derived expected body mass, according to female size and chick age.

Colour measurements

We measured the colour of the female head and tarsus based on digital photos. We used a Canon EOS 350D digital camera (8.0 megapixels) with a Canon Zoom Lens (EF-S 18–55 mm f/3.5–5.6) with automatic settings for integration time (shutter speed) and lens aperture, and with the white balance set to 'daylight'. All photos were taken outdoors with the integrated flash lighting. We held the right tarsus next to a yellow reference card close to the ground and took two pictures from above (Fig. S2). Similarly, a photo of the head together with an orange reference card was taken from the left side (Fig. S3). We avoided direct sunlight on tarsus, head and reference card. Our method of colour measurement only includes the human-visible spectrum (approximately 400–700 nm) and excludes variation in the ultraviolet range, which is visible to the birds (e.g. Cuthill et al. 2000). However, the analysis of digital photos has proved to be a useful tool to study colouration in animals in general (Bergman and Beehner 2008) and also for the study of carotenoid based colouration in birds (e.g. Fitze et al. 2003; Perez-Rodriguez 2008; Mougeot et al. 2009).

Each digital photograph was imported into Adobe Photoshop CS3, and colour was measured as follows. First, we randomly selected five non-shaded areas of 100×100 pixels along the tarsus between the tibio-tarsal articulation and the foot and five non-shaded areas on the head in a circle around the eye and recorded the red, green and blue (RGB) levels using the histogram palette (averaged over the selected pixels) following Fitze and Richner (2002). Similarly, we measured five areas of the same size on the reference cards, and we calculated a mean value for tarsus, head and reference card. Digital images consist of a matrix of microscopic photocells where colour is recorded as brightness values (in the range, 0–255) of RGB. If any of the RGB values for any square was 255 (the upper limit of the camera), the photo was discarded (see Bergman and Beehner 2008). Because the actual value in each channel is only informative relative to the values in the other channels, we analysed the ratio of red to green (R/G) (Bergman and Beehner 2008). To account for differences of natural lighting conditions, we used the difference between the tarsus R/G value and the R/G value of the yellow reference card and the difference between the head R/G value and the R/G value of the orange reference card in each picture for further analysis and refer to this as 'R/G' for simplicity. R/G values of the two pictures taken in row were highly correlated ($r=0.88$, $n=64$, $P<0.001$), and we used the mean value of the two pictures for further analysis.

To better describe what R/G difference values signify in terms of colour, we assessed the colour of our reference card using the GretagMacbeth ColorChecker colour rendition chart [product no. 50105, manufactured by Munsell Colour, division of GretagMacbeth; for a detailed description and picture of the chart see Bergman and Beehner (2008)]. We took 20 photos of the ColorChecker together with the yellow and orange reference card, respectively, and analysed them in Photoshop, as described above. We calculated the differences between the R/G values of the yellow card and the R/G values of the orange, orange-yellow and yellow square on the ColorChecker chart (R/G difference = value ColorChecker − value reference card). The difference increased from yellow (−0.335±0.010), over orange-yellow (0.008±0.015) to orange (0.860±0.033); that is, higher tarsus R/G values indicate a more orange-like tarsus in our study. The same was done for the orange reference card, where we calculated the differences to the red, orange and orange-yellow square. Here, the difference increased from red (−2.750±0.232) over orange (0.965±0.091) to orange-yellow (1.683±0.091); that is, lower head R/G values indicate a more red-like head colouration in our study.

Carotenoid analysis

We quantified plasma carotenoid levels following Alonso-Alvarez et al. (2004). We diluted 10 µl of plasma in 90 µl of absolute ethanol, vortexed the mixture and centrifuged it at 1500×g to precipitate flocculent proteins. The supernatant was examined in an Ultrospec 2000 (Pharmarcia Biotech) spectrophotometer, and the optical density at 450 nm (maximal absorbance of lutein) was determined. We calculated plasma carotenoid concentration (microgram per millilitre) using a standard curve of lutein (Sigma Chemicals). Each plasma sample was double tested, and the obtained values were highly correlated ($r=0.97$, $N=83$, $P<0.001$). Alonso-Alvarez et al. (2004) assessed the reliability of this technique by comparing a high performance liquid chromatography analysis of plasma samples with colorimetric measurements. HPLC determined the presence of four carotenoids in the plasma (lutein, zeaxanthin, anhydrolutein and β-cryptoxanthin). The total amounts of carotenoids determined by the two techniques were highly correlated; colorimetric measurements could thus be considered as representative of total plasma carotenoid concentration.

Statistical analysis

Statistical tests were performed with SPSS 11.0. Normality was tested with Kolmogorov–Smirnov tests. Means are given with standard errors. We used Pearson correlations and stepwise linear regressions if the assumptions of normality and equal variances were met. Significance level was set at $\alpha=0.05$.

We captured and measured 29 females in 2007 and 38 in 2008, of which 20 females were measured in both seasons. To test which parameters (colouration, body condition, PC1 as a measure of body size and hatching date) have the biggest influence on clutch and egg size, we performed stepwise linear regressions, with probability of 0.05 for entry and 0.10 for removal. We only included the first measurement of each female in these analyses to avoid pseudoreplication. To test whether tarsus and head colouration could be predicted from body condition, body size and hatching date, we also performed stepwise linear regressions. As captured adult birds were of unknown age, individual age could not be included in the analyses. Sample sizes differed because of missing values. Some territories were not visited before chicks hatched, and hence, data on clutch size and egg volumes are missing. Furthermore, not all females were blood-sampled, and some photos were discarded (see above).

Results

The tarsus and head R/G values were similar for the two study years (Table 1). Tarsus and head R/G values were not correlated (Pearson correlations, $r=0.063$, $N=40$, $P=0.700$).

Tarsus colouration, reproductive parameters and body condition

Stepwise multiple regression suggested that clutch volume increased significantly with tarsus R/G values, with females with more orange-like tarsus colouration having laid clutches with a bigger volume, and was higher in individuals with earlier hatching dates (Table 2 and Fig. 1a). In addition, mean egg volume was higher in individuals with higher tarsus R/G values (Table 2 and Fig. 1a). Stepwise multiple regression found none of the possible explanatory variables body condition, body size and hatching date to be related to variation in tarsus R/G values.

Head colouration, reproductive parameters and body condition

Stepwise multiple regression revealed that clutch volume was not related to head R/G values and body size but could be best predicted from a combination of hatching date and body condition (Table 2). Mean egg volumes were higher in females with a more red-like head colouration (Table 2 and Fig. 1b). Head R/G values were lower, i.e. more red-like, in females with a higher body condition ($F_{1,40}=7.442$, $t=-2.728$, $P=0.010$, Fig. 2) but unrelated to body size and hatching date.

Tarsus and head colouration and plasma carotenoid concentrations

The concentration of carotenoids circulating in the plasma ranged from 3.1 to 31.5 µg/ml and was similar for the two study years (Table 1). Plasma carotenoid concentrations were independent of capture date ($r=-0.03$, $P=0.78$, $N=79$).

Plasma carotenoid concentrations were higher in females with higher R/G values, i.e. a more orange-like tarsus colouration (Fig. 3, Pearson correlation: $r=0.336$, $P=0.015$, $N=52$) but unrelated to head colouration ($r=-0.091$, $P=0.587$, $N=38$). Plasma carotenoid concentrations were also higher in females with higher mean egg volume but unrelated to clutch volume (Pearson correlations; mean egg volume, $r=0.50$, $P=0.008$, $N=27$; clutch volume, $r=0.31$, $P=0.120$, $N=26$; Fig. 4). The results remained qualitatively the same after controlling for hatching date (partial correlations; mean egg volume: $r_p=0.46$, $df=23$, $P=0.020$; clutch volume: $r_p=0.22$, $df=23$, $P=0.290$).

Individual comparisons across years

Plasma carotenoid concentrations were significantly correlated between the two study years (Fig. 5a, $r=0.56$, $N=20$,

Table 1 Mean values of plasma carotenoid concentrations and tarsus and head colouration of adult female upland geese in the wild: we tested for differences between the years using analysis of variance (see "Statistical analysis" section)

		Mean ± SE	Min	Max	*n*
Carotenoids	2007	17.47±1.11 µg/ml	3.06 µg/ml	28.77 µg/ml	35
	2008	15.46±0.85 µg/ml	6.05 µg/ml	31.48 µg/ml	47
	Test	$F_{1,80}=2.15$, $P=0.146$			
Tarsus R/G	2007	−0.068±0.017	−0.270	0.120	29
	2008	−0.037±0.014	−0.190	0.220	38
	Test	$F_{1,65}=2.04$, $P=0.158$			
Head R/G	2007	−1.391±0.053	−2.000	−0.900	23
	2008	−1.439±0.047	−2.200	−0.110	28
	Test	$F_{1,50}=0.431$, $P=0.515$			

Table 2 Results of stepwise multiple linear regression models for clutch and mean egg volume, including tarsus and head colouration of adult female upland geese as predictor variables (n.i. means that the variable was not included by the model as a significant predictor)

	Clutch volume	Mean egg volume
Model tarsus	$F_{2,19}=9.305$, $P=0.002$	$F_{1,20}=7.015$, $P=0.016$
Tarsus R/G	$t=3.103$, $P=0.006$	$t=2.649$, $P=0.016$
Hatching date	$t=-2.502$, $P=0.023$	n.i.
Body condition	n.i.	n.i.
Body size (PC1)	n.i.	n.i.
Model head	$F_{2,16}=6.845$, $P=0.008$	$F_{1,17}=4.864$, $P=0.042$
Head R/G	n.i.	$t=-2.205$, $P=0.042$
Hatching date	$t=-2.539$, $P=0.024$	n.i.
Body condition	$t=2.210$, $P=0.044$	n.i.
Body size (PC1)	n.i.	n.i.

$P=0.01$). The intra-individual differences ranged from 0 to 12 μg/ml with a mean of 5.40±0.7 μg/ml. In addition, the tarsus colour (R/G values) of individuals measured in 2007 and in 2008 was significantly correlated among years (Fig. 5b, $r=0.65$, $N=15$, $P=0.009$), but head R/G values were not ($r=0.61$, $N=8$, $P=0.112$). The individual changes in tarsus R/G values (value 2008−value 2007) were significantly positively related to differences in carotenoid concentrations between the two study years (Fig. 6, Pearson correlation: $r=0.52$, $N=15$, $P=0.048$).

Discussion

We found that female-specific colouration in upland geese can reliably indicate different aspects of female phenotypic quality. Females with more orange coloured legs and more red-like head colours had higher clutch and egg volumes than females with a paler leg and head colouration. Furthermore, individuals with a more reddish plumage colouration were in a better body condition. These relationships provide the theoretic possibility for males to assess female phenotypic quality on the basis of colouration. A male mated with a female with more orange coloured legs and more reddish head plumage can expect a higher reproductive output. To determine whether male mate choice is influenced by colouration, however, experiments have to be carried out. We also found that female tarsus colour was repeatable between years, indicating that tarsus colour is a reliable signal of long-term female quality in this species. At the same time, tarsus colouration was also a dynamic trait because the variability in tarsus colour reflected differences in plasma carotenoid levels both among and within individuals.

Egg size has been discussed in the context of individual quality before, with phenotypic and genetic factors predisposing some individuals to perform better under a given set of conditions and lay larger eggs (Reid and Boersma 1990; Croxall et al. 1992; Blackmer et al. 2005; Ardia et al. 2006). Especially in precocial species like waterfowl,

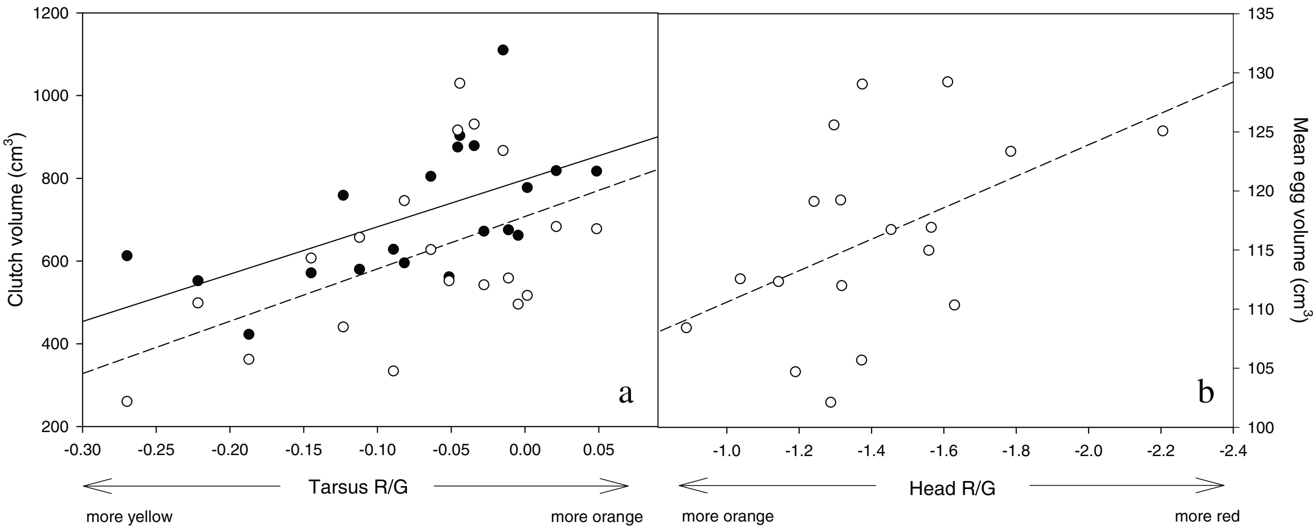

Fig. 1 Relationship between **a** tarsus colour and **b** head colour (R/G values) and clutch volume (*black dots*, *solid line*) and mean egg volume (*white dots*, *dashed line*) in female upland geese on New Island. Please note the inverse scale for head R/G values. See text for statistical analysis

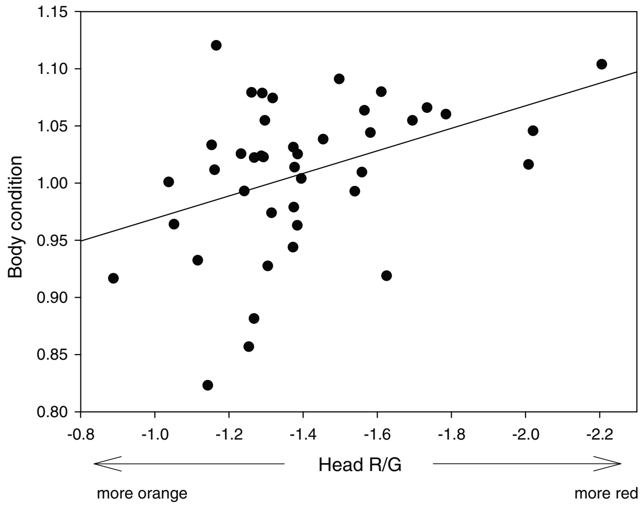

Fig. 2 Relationship between head colour (R/G values) and body condition in female upland geese on New Island. Please note the inverse scale for head R/G values. See text for statistical analysis

female egg investment may play an important role in determining survival prospects of offspring (Amat et al. 2001) and thereby increasing individual fitness. Larger eggs may enhance offspring fitness by (a) increasing survival prospects in the first days after hatching and (b) increase competitive ability through larger offspring size and the possession of more resources to survive adverse conditions (Ankney 1980; Amundsen and Stokland 1990; Magrath 1992; Dawson and Clark 2000; Anderson and Alisauskas 2002; Christians 2002; Goth and Evans 2004; Rutkowska and Cichon 2005). In upland geese, body condition of young of age ≤20 days increased with increasing egg size Gladbach et al. (2010). The positive relationship between female colouration and egg and clutch size in our study system may therefore indicate individual reproductive quality. Furthermore, we found that female tarsus colouration is positively related to plasma carotenoid levels, suggesting that it is an honest indicator of the health state or of immunocompetence in female upland geese. This is in line with other studies showing that integument colouration reflected circulating carotenoid levels (Juola et al. 2008; Perez-Rodriguez 2008; Casagrande et al. 2009; Mougeot et al. 2009).

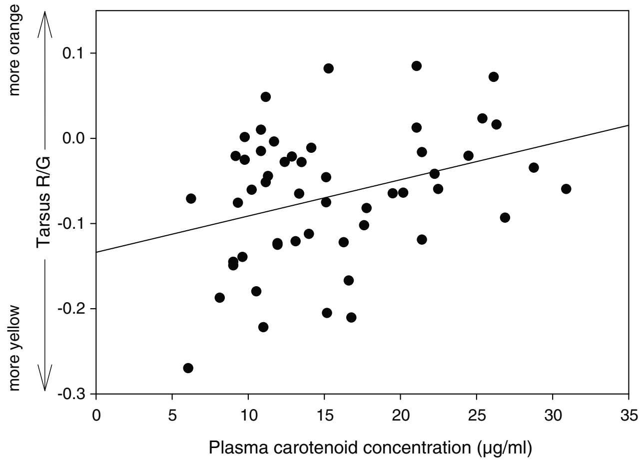

Fig. 3 Relationship between plasma carotenoid concentrations and tarsus colour (R/G values) in female upland geese. See text for statistical analysis

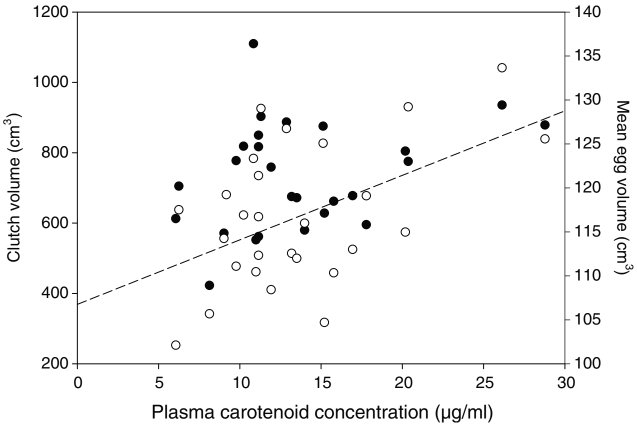

Fig. 4 Relationship between plasma carotenoid concentrations and clutch volume (*black dots*) and mean egg volume (*white dots*, *dashed line*) in female upland geese. See text for statistical analysis

Only few studies have shown that a strongly environmentally determined signal like carotenoid-based integument colouration is consistent within individuals over time (Dawson and Bortolotti 2006; Perez-Rodriguez 2008). Such consistency is important in relation to male mate choice, especially in species that form life-long pair bonds such as the upland goose. If a sexual trait changes over time, the trait might be honest, but it would only reflect the current state and would have little predictive value for future reproductive success (Greenfield and Rodriguez 2004; Senar and Quesada 2006; Perez-Rodriguez 2008). Dawson and Bortolotti (2006) found in female American kestrels *Falco sparverius* that integument colour scores in the pre-laying period and during incubation were correlated. Perez-Rodriguez (2008) reported that in red-legged partridge *A. rufa*, bill and eye-ring colouration were highly repeatable within individuals, both within the breeding season and between two consecutive years. We show here that tarsus colouration of the same individuals in two consecutive years is strongly correlated. Measures of individual quality should be relatively consistent over longer time spans to be a reliable indicator for the inherent quality of individual birds (Ochs and Dawson 2008); female tarsus colouration thus could be used as such a quality signal. Monitoring over several years together with a complete data set for measures of phenotypic quality could shed further light on the usefulness of tarsus colouration as a measure of individual quality in female upland geese. We also found a strong

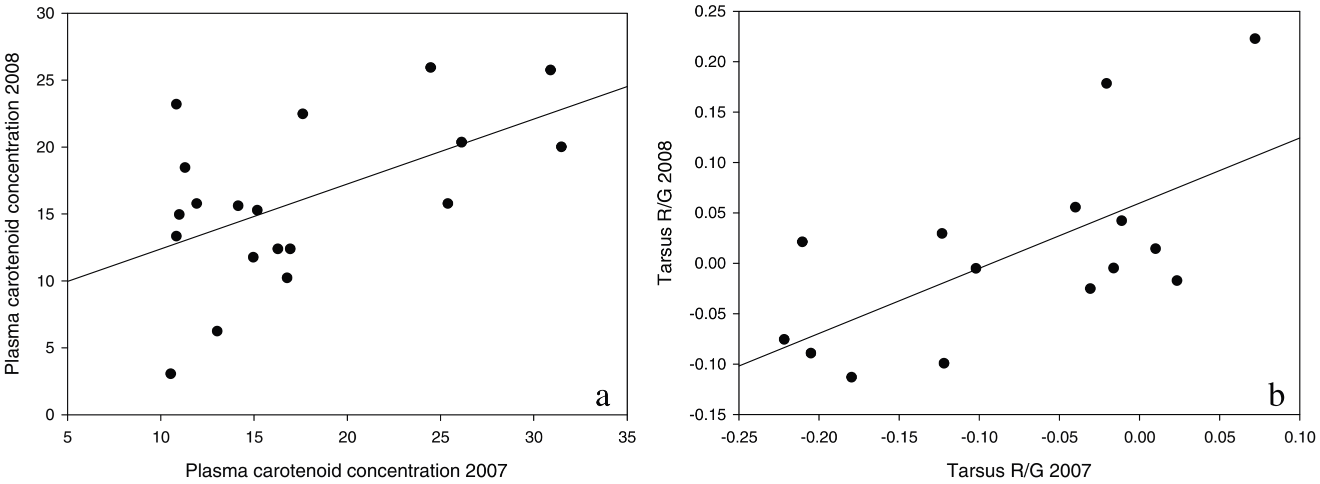

Fig. 5 Relationship between **a** plasma carotenoid concentrations and **b** tarsus colour (R/G values) of the same individual female upland geese measured in subsequent years

correlation between years in individual plasma carotenoid concentrations. This contrasts with the study of Perez-Rodriguez (2008), where no such correlation was found. However, in that study, birds were kept in an aviary and fed ad libitum. Variability between individuals even when carotenoid access and health status are standardised indicates that intrinsic physiological or genetic factors may affect the ability to absorb, transport and transform carotenoids (McGraw and Hill 2001; Hadfield and Owens 2006). In our study, within-individual variation (maximum difference, 12 µg/ml) was smaller than between individual variation (maximum difference, 29 µg/ml). Despite relatively small intra-individual variation, the changes in plasma carotenoid levels were still reflected in individual variation in tarsus colouration between the 2 years. Our data thus support results from aviary experiments (Perez-Rodriguez 2008) and show that integument colouration is a dynamic trait remaining consistent between individuals also in a wild bird under natural conditions.

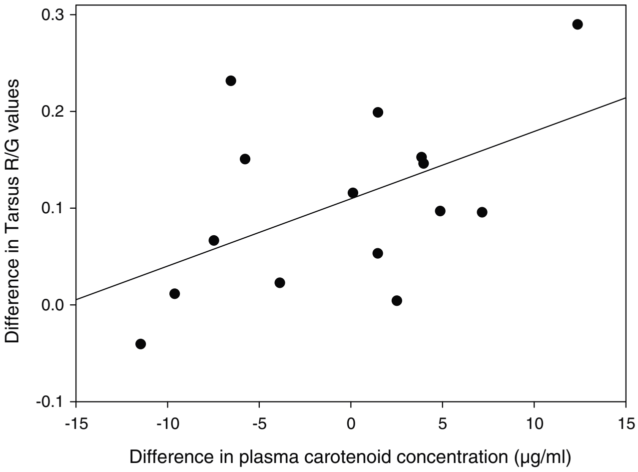

Fig. 6 Relationship between the change in plasma carotenoid concentrations and the change in tarsus colour (R/G values) between two consecutive years (2007–2008) in female upland geese

Territory quality and carotenoid availability in territories may be an important factor in determining the between-year correlation in plasma carotenoid levels. This is because upland geese are highly territorial and return to the same territories year after year. Measurements of tarsus colour and plasma carotenoid levels during the non-breeding season, when upland geese gather in large groups around ponds and thus share the same environmental conditions, could shed further light on the importance of the breeding territory for the expression of the ornament. Furthermore, we cannot exclude the hypothesis that females mated to high-quality males that defend high-quality territories have more orange-like tarsi because they would have access to carotenoid-rich food and are able to invest more in reproduction. This could be studied experimentally, by removing pairs and measuring changes in tarsus colour in relation to changes in territory quality. This would also require that at least territory quality and perhaps also male competitive ability in territory defence can be measured. Preliminary, we found no relationship between male body condition and female tarsus colouration and no repeatability of male plasma carotenoid concentrations between two consecutive years Gladbach et al. (2010), making this explanation perhaps less likely.

The reddish head colouration in upland geese may be based on melanins. Melanins are one of the most important groups of pigments involved in bird colouration (McGraw 2006) and responsible for reddish-brown, brown, black or grey colour of feathers. The effects of environmental and individual quality on melanin-dependent traits are still discussed (Griffith et al. 2006; Ducrest et al. 2008; McGraw 2008). Several studies have found melanin-based traits to be related to individual quality (reviewed in Jawor and Breitwisch 2003). Melanogenesis can be influenced by physiological condition with food availability and changing hormonal conditions affecting the biosynthesis and deposition of melanin into ornaments (Jawor and Breitwisch 2003), which could explain the more reddish head colouration of females with higher body condition. Alternatively, this may also be linked to a condition dependency of the moult of body feathers, with females in lower body condition skipping moult. The skipping of moult of primaries in sometimes three consecutive years has been reported in upland geese before (Summers 1983b), and the gradual impairment of flight feathers might be unimportant to this non-migratory species. This moult skipping could have the advantage to save energy and nutrients, which possibly also applies to body feathers. The colour of feathers might then be subject to degradation, leading to a paler colour of older feathers. This is supported by one case where we caught a female moulting head feathers (not included in the analyses) and the new feathers were of a darker reddish-brown colour than the old ones (Fig. S4). Another possibility is the occurrence of an age effect, with older females producing darker feathers. Both increases (e.g. Dale et al. 2002; e.g. Galvan and Moller 2009; Vergara et al. 2009) and decreases (e.g. Potti and Montalvo 1991; Siefferman et al. 2005) in the intensity of melanin-based colour with age have been reported before. To test this in upland geese, a comparison of individual feather colouration in several years or between individuals with known age is necessary. The non-significant correlation of head colour between the 2 years could be caused by the small sample size in our study (only pictures of eight females could be compared) because the high correlation coefficient (0.61) indicates that there is a trend also for head colouration to be stable between years. Plumage characters have been found to remain reasonably stable between moults in other studies (e.g. Senar and Quesada 2006). However, to confirm this in upland geese, the comparison of a larger number of individuals is necessary.

In summary, our study shows evidence that the yellow-orange tarsus and reddish-brown head in female upland geese, a colouration that only occurs in females, can indicate individual quality. Only a small number of previous studies reports positive relationships between female-specific ornamentation and aspects of phenotypic quality (Domb and Pagel 2001; Weiss 2006; Weiss et al. 2009). Jawor and Breitwisch (2003) hypothesise that birds displaying both carotenoid and melanin ornaments provide more reliable indication of their overall quality than birds displaying only one or the other type of ornament. We furthermore report for the first time in a wild bird under natural conditions that a female-specific ornament is stable across years. Following individuals for a longer period of time and including birds of known age (ringed as chicks) would further add to our knowledge about the development and reliability of quality signals in birds establishing long-term pair bonds.

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Ethical note Families were caught in their territories when leading broods using a whoosh net. Adult birds were handled first, and chicks were released back to their families after handling, who accepted them back readily. The study was carried out with the permission of the Environmental Office of the Falkland Islands. Upland geese are not protected and can be captured/disturbed at any time.

Conflict of interest The authors declare that they have no conflict of interest.

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SEVEN

Male achromatic wing colouration is related to body condition and female reproductive investment in a dichromatic species, the Upland goose

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ARTICLE

Male achromatic wing colouration is related to body condition and female reproductive investment in a dichromatic species, the upland goose

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Abstract In many bird species, achromatic plumage patch size can serve as a male status signal, but the use of variations in the achromatic colours themselves as a quality signal has only recently come into focus. In our study, we sought to determine whether achromatic plumage reflects individual quality in the upland goose (*Chloephaga picta leucoptera*). We examined the relationship between male head and wing reflectance, male condition and female reproductive investment. We found that males with darker specula and greater contrast between the white wing coverts and the speculum were in a better body condition. Variations in the brightness of the white plumage were not a quality signal in the upland goose. The information gleaned from the wing colouration of male upland geese could be used during mate selection, when females choose their mate on the basis of the outcomes of aggressive encounters. During these fights, the males expose their white coverts and their specula, which are normally tucked beneath body feathers.

Keywords Upland goose · *Chloephaga picta leucoptera* · Male colouration · Individual quality · Achromatic colour · Speculum

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Introduction

In many species, females prefer to mate with males with the most elaborate ornaments (Andersson 1994), as those traits can indicate aspects of male quality (Zahavi 1975; Kodric-Brown and Brown 1984). In birds, many studies have found plumage colouration to be correlated to different male quality characteristics, like condition (e.g. Keyser and Hill 1999; Peters et al. 2007), territory quality (e.g. Keyser and Hill 2000), parasite load (e.g. Hõrak et al. 2001), immune function (e.g. Maney et al. 2008) or parental effort (e.g. Keyser and Hill 2000; Siefferman and Hill 2003).

Achromatic plumage colouration (black, grey and white) has been studied before, with the main focus being the patch size of achromatic plumage, including its relation to reproductive investment, individual quality and status signalling (e.g. Moller 1987; Part and Qvarnstrom 1997; e.g. Doucet et al. 2005; Hanssen et al. 2006; Hanssen et al. 2008). Characteristics of the achromatic colour itself (like brightness and chroma) as a quality signal have only come into focus quite recently (e.g. Mennill et al. 2003; Woodcock et al. 2005; Hanssen et al. 2009), although there has been evidence from observations and experiments that female choice may be influenced by variation in achromatic colour (e.g. Saetre et al. 1994). For example, in male black-capped chickadees *Poecile atricapillus*, reproductive success was predicted by the reflectance of achromatic plumage (Doucet et al. 2005), and in the ostrich *Struthio camelus*, male white colouration was found to be related to male immune capacity (a measurement of male quality), and both black and white colouration to egg mass, indicating that females adjust their investment according to their perception of male quality (Bonato et al. 2009a, b).

In our study we sought to determine whether achromatic plumage characteristics reflect individual quality in the upland goose *Chloephaga picta leucoptera.* Upland geese belong to the order of the sheldgeese (Tadornini), a group that resembles true geese and shows similar habits but is more closely related to shelducks and ducks. One of the most interesting aspects of the closely knit group of the genus *Chloephaga* is the great variation in colouration between the species and sexes, ranging from nearly no dimorphism in the Andean goose *C. melanoptera* (both sexes are mainly white), the ruddy-headed goose *C. rubidiceps* and the ashy-headed goose *C. poliocephala* (both have mainly brown plumage), to the kelp goose *C. hybrida* and the upland goose, which exhibit very strong sexual dimorphism, with males being mainly white and females brown (Summers and McAdam 1993). While this dimorphism is still restricted to the plumage in kelp geese, it even extends to the integument colouration in upland geese (males: black tarsus, females: yellow-orange tarsus). Besides this, males and females share some wing characteristics, namely white upper wing coverts, a metallic-coloured speculum formed by the secondary coverts (rather than by the secondaries, as in other anatines), and white secondaries. There is still speculation about the causes of the differences in white colouration in this group, with one possibility being that it evolved in order to be conspicuous (Summers and McAdam 1993). Especially kelp and upland geese are very aggressive birds, defending large territories and signalling their ownership with erect posturing. A plumage colouration that contrasts as much as possible with the background would thus be advantageous. In females, the need to be cryptic during incubation could have preserved the brown plumage. However, this theory cannot explain the white plumage in female Andean geese. Upland geese are highly territorial and socially monogamous, usually returning to the same territory with the same mate every year (Summers and McAdam 1993). Male upland geese invest heavily in the establishment and defence of their breeding territory. They increase their aggressive activities gradually during the season. Besides evicting their own young from the previous breeding season, the territory has to be defended against intruding pairs looking for a breeding place, and territorial boundaries with neighbouring pairs need to be maintained throughout the breeding season (Summers and McAdam 1993). Aggressive encounters range from pacing side by side along the territorial boundaries, the exposure of their carpal knobs, to gripping each other by the neck and dashing their wings against each other. When attacking another bird, the male runs rapidly over the ground with its head low and his folded wings slightly spread, thus exposing his white upper coverts and his speculum, which are both normally hidden when the wing is closed [Electronic supplementary material (ESM), Fig. S1].

White plumage may be used in signalling in different ways: either as a quality signal itself or as a contrast to coloured plumage parts. Despite being inexpensive to produce, as the feathers contain no costly pigments, white plumage may serve as a quality signal, with costs arising from its maintenance and the fact that feather quality is more visible (Fitzpatrick 1998). Depigmented feathers have been found to wear faster in birds with lower feather quality, thereby indicating a birds' ability to invest in high-quality feathers (e.g. Kose and Moller 1999). Additionally, the contrast of the white with the background or dark plumage parts may be an important signal, as it can improve the perception of displays or the signaller itself (Galvan 2008). To investigate whether white plumage may serve as a signal of quality in male upland geese, we examined the relationship between achromatic plumage reflectance, male condition, and female reproductive investment. Additionally, we compared the possible signalling function of wing colouration between males and females.

Study area

The study was carried out in the New Island Nature Reserve, Falkland Islands (51°43′S, 61°17′W), from October to December in 2007 and 2008. New Island was established as a nature reserve in 1973, when all livestock were removed from the island. This led to an increase in the density of upland geese on this island, making it one of the most densely populated of the Falkland Islands (Quillfeldt et al. 2005).

Materials and methods

The basic breeding biology and life cycle of upland geese was studied in the Falklands from 1977 to 1980 (Summers 1983a). Take-up of territories starts in August, and egg laying occurs in late September, with most clutches being laid by the end of October. Hatching mainly takes place between mid-October and mid-November, and fledging starts at approximately 70 days of age.

At the beginning of each field season, the nests were mapped. Numbers of eggs per nest were counted, length and breadth of eggs were measured to the nearest 0.1 mm using calipers, and weight was recorded to the nearest 0.1 g using a digital balance. Egg volume (V) was determined from egg length L (in cm) and egg breadth B (in cm) as $V = (L \times B^2 \times 0.507)$, according to Furness and Furness (1981). Total volume of the clutch was determined by summing the individual volumes of all eggs in each clutch.

We caught adults during the period when they attended their brood using a 3 × 5 m whoosh net. One person herded the family of geese slowly to the catching area, and when they arrived directly in front of the furled net, the other researcher pulled the trigger. Adults were marked with individual metal rings and weighed to the nearest 10 g using a digital spring balance. Head length, culmen length, and tarsus length were measured to the nearest 0.1 mm using callipers; wing length (maximum flattened chord) was measured to the nearest 1 mm using a stopped rule. A principal components analysis (PCA) extracted one principal component (PC1) from wing, head, bill and tarsus measurements as an overall measure of body size in both males and females. In females, PC1 (with an eigenvalue of 1.794) explained 44.844% of the variance. In males, PC1 (with an eigenvalue of 2.001) explained 50.018% of the variance. The body condition for males and females was determined while accounting for structural size, based on a regression of body mass on PC1. Body condition was expressed as the ratio of the observed body mass to the derived expected body mass, according to individual size.

Spectrophotometric colour measurements

Reflectance spectra between 300 and 700 nm were recorded using an AvaSpec-2048 fibre optic spectrometer (Avantes, Eerbeek, The Netherlands) with an AvaLight-DHc deuterium/halogen light source unit, and FCR-7UV400 reflection probe fibre optics. We took five readings each from the white head, the white wing coverts and the speculum on each male. As each trait appeared uniform in colour, it was measured in randomly allocated places, moving the probe at least 2 cm between measurements. We used the average reflectance curve from the five readings for each region in the following analyses. Reflection was recorded using a probe held normal to the surface, collecting light from a spot 4.5 mm in diameter. The measurements were taken outside with the probe held directly against the feather surface, so that no ambient light would affect the measurements. A white reference (WS-2 white reference tile for reflectance measurements, Avantes) and a dark reference (measured with the lights turned off) were taken for calibration purposes before each individual was measured.

Spectrometric data were analysed using AVICOL v.3 (Gomez 2006). Brightness was calculated as the mean reflectance between 300 and 700 nm. To describe the contrast between the white wing coverts and the speculum, we calculated the contrast as the ratio of the mean brightness values (% reflectance) of the wing coverts to the speculum. We also measured aspects of the UV colouration of the speculum (UV brightness, UV chroma and maximum reflectance between 350 and 450 nm). UV brightness was highly correlated with the overall brightness of the speculum ($r = 0.961$, $P < 0.001$), as were UV contrast and overall contrast ($r = 0.947$, $P < 0.001$).

Statistical analysis

Statistical tests were performed in SPSS 11.0. Means are given with standard errors. The significance level was set to $P < 0.05$. Normality was tested using Kolmogorov–Smirnov tests. We assessed significance with Pearson correlations and analysis of variance (ANOVA). We captured and measured 32 females and 34 males in 2007 and 46 females and 35 males in 2008, of which 20 females and 10 males were measured in both seasons. We found no differences between the study years in both males and females in all measured parameters (all $P > 0.200$). We therefore decided to pool all data in the subsequent analyses and use the first measurement of each animal to avoid pseudoreplication. Note that sample sizes may differ because of missing values. Some territories were not visited before the chicks had hatched, and hence data on clutch size and egg volumes are missing.

Results

Differences between the sexes

Females had brighter white wing coverts than males (Table 1), but we found no sex differences in the brightness of the speculum, speculum UV characteristics, and in the contrast between wing coverts and the speculum (Table 1).

Colouration and body condition

Body condition was higher in males with lower brightness values of the speculum ($r = -0.37$, $N = 55$, $P = 0.006$; Table 2; Fig. 1a) and lower UV brightness of the speculum ($r = -0.31$, $N = 55$, $P = 0.020$), but it was unrelated to the brightness of the head and the wing coverts (Table 1). We found that the contrast between the wing coverts and the speculum (brightness of wing coverts/brightness of speculum) was significantly related to body condition, with males that had higher ratios (i.e. darker specula compared to wing coverts) being in better body condition ($r = 0.48$, $N = 55$, $P < 0.001$; Table 2; Fig. 1b). In females, we found no significant relation between wing colouration and body condition (all $P > 0.1$).

Colouration and reproductive parameters

In males, the average brightness of the speculum was negatively related to clutch volume, with individuals with a darker speculum being mated to females that laid larger

Table 1 Mean reflectance values (%) of wing coverts and the speculum, UV chroma and UV peak of the speculum, and mean contrast between wing coverts and the speculum of upland geese in the wild

	Males			Females			Test
	Mean + SE	Range	*n*	Mean + SE	Range	*n*	
Wing coverts	98.24 ± 1.34	69.51–120.38	55	103.05 ± 1.31	103.05–1.31	60	$F_{1,114} = 6.558$, $\boldsymbol{P = 0.012}$
Speculum	13.66 ± 0.46	6.65–22.35	55	13.43 ± 0.48	7.78–25.56	60	$F_{1,114} = 0.116$, $P = 0.734$
Speculum UV brightness	12.67 ± 0.45	5.59–20.45	55	12.83 ± 0.45	6.84–24.57	60	$F_{1,114} = 0.063$, $P = 0.802$
Speculum UV chroma	0.23 ± 0.01	0.19–0.27	55	0.24 ± 0.01	0.19–0.26	60	$F_{1,114} = 0.326$, $P = 0.569$
Speculum UV peak	406.76 ± 3.64	351–450	55	405.76 ± 3.73	350–450	60	$F_{1,114} = 0.036$, $P = 0.849$
Contrast between wing coverts and speculum	7.58 ± 0.24	4.27–12.16	55	8.18 ± 0.28	4.00–14.39	60	$F_{1,114} = 2.691$, $P = 0.104$

We tested for differences between the sexes using analysis of variance (ANOVA). Significant value is stated in bold

Table 2 Relationships between the brightnesses of different plumage regions and body condition, clutch and mean egg volume in male upland geese on New Island

Body region	Body condition	Clutch volume	Mean egg volume
Head	0	0	0
Wing coverts	0	0	0
Speculum	–	–	0
Contrast between wing coverts and speculum	(+)[a]	0	0

Pearson correlations: “0”: no significant effect (i.e. $P > 0.05$), “+” positive relationship (i.e. increase or decrease together), “−” negative relationship (i.e. one increases while the other decreases)

[a] The positive relation between clutch volume and speculum was no longer significant at the 0.05 level after controlling for hatching date. There were no significant relationships in females. See the “Results” section for statistics

clutches (Pearson correlation: brightness: $r = -0.41$, $N = 24$, $P = 0.049$; Table 1; Fig. 2, UV brightness: $r = -0.44$, $N = 24$, $P = 0.039$). As there is a seasonal trend (with clutch volume being higher in earlier clutches; Gladbach et al. 2010b), we ran a second analysis controlling for hatching date (partial correlation: brightness: $r = -0.326$, $N = 24$, $P = 0.129$; UV brightness: $r = -0.30$, $N = 24$, $P = 0.159$). The mean egg volume was unrelated to male colouration. There was no significant relationship of wing colouration to clutch volume or mean egg volume in females, although brightness of the speculum showed a tendency to be negatively related to clutch volume ($r = -0.290$, $N = 23$, $P = 0.180$).

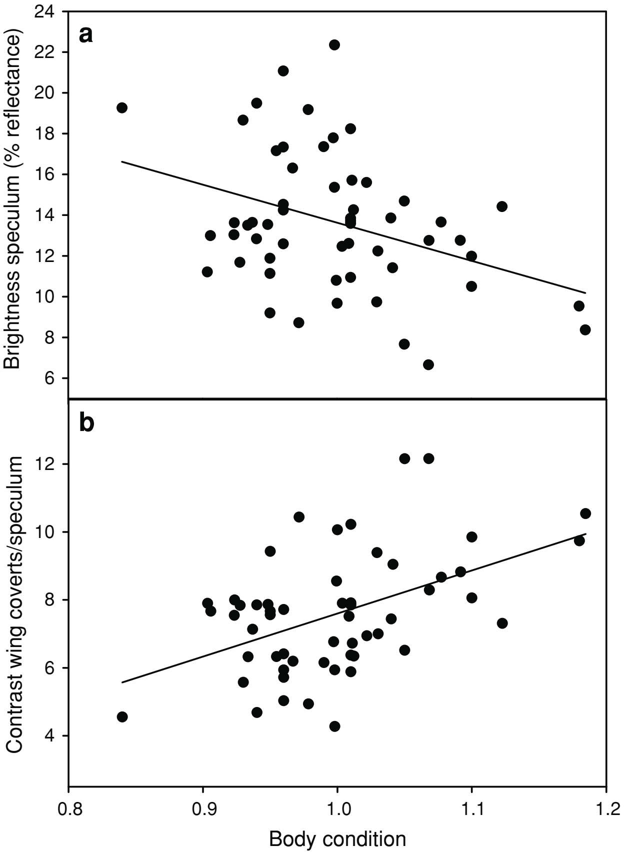

Fig. 1 Relationship between brightness of the speculum and body condition (**a**) and relationship between and body condition and contrast between wing coverts and speculum (**b**) in male Upland geese measured on New Island. See text for statistical analysis

Discussion

Our findings demonstrate that in upland geese, male achromatic plumage reflectance predicts body condition in males and investment in clutch volume by their pair partners. In particular, we found that males with darker specula and a greater contrast between their white wing coverts and their specula had better body conditions. Females mated to males with darker specula laid clutches with bigger volumes. We could not support the hypothesis that variations in the brightness of the white plumage itself

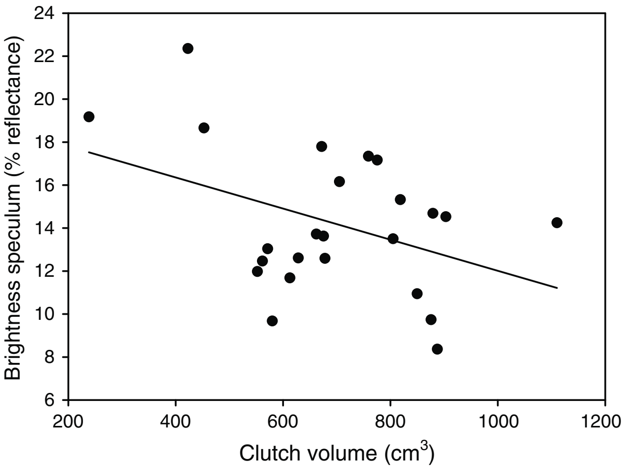

Fig. 2 Relationship between brightness of the speculum of male upland geese and the clutch volume laid by their partner on New Island. See text for statistical analysis

are a quality signal in the upland goose. Males and females only differed in the brightness of their wing coverts, which may be explained by the greater wear of these feathers in males where they are exposed during fights, which is not the case for females. We also found no relation of most of the UV parameters of the speculum to either body condition or reproductive parameters in males and females. Only the UV brightness of the speculum in males was related to body condition, but as UV brightness and overall brightness were highly correlated, we would be cautious to interpret this finding as UV signalling.

The information content of the wing colouration of male upland geese could be used during mate choice. During pair formation, females incite males to attack other males or females, and then select their mate on the basis of the outcome of these aggressive encounters (Johnsgard 1965). The male responds to female incitation by adopting an erect posture, holding the carpal joints of the wings out from the body, thereby exposing the white coverts and the speculum, which are normally tucked beneath body feathers (ESM Fig. S1). Avian wing displays can signal aggressiveness or willingness to escalate fights (Hurd and Enquist 2001), and the white coverts and the speculum in the upland goose may function as “coverable badges” (Hansen and Rohwer 1986) that are only exposed when birds are highly motivated to fight but are hidden when the birds are not willing to engage in an aggressive encounter. Differences in male wing colouration may serve as an indicator of status or condition, and reduce the need for costly aggressive interactions. Based upon our results, it can be speculated that not only the outcome of the aggressive encounters but also aspects of plumage quality may influence female choice in the upland goose.

Contrasts between adjacent plumage regions have been found to be an important aspect of achromatic plumage in some other studies (Mennill et al. 2003; Bokony et al. 2006; Bonato et al. 2009a). For example, Bokony et al. (2006) could show that in male house sparrows (*Passer domesticus*), the conspicuousness of wingbars may increase the defence success of their bearer in aggressive interactions. Measurements of male plumage characteristics during the time of pair formation and their relation to lost/won fights could further highlight the role of such signals in the upland goose. To our knowledge, there have been no studies so far on the signalling ability of the iridescent speculum found in the Anatidae. Iridescent colours change in appearance with the angle of observation or illumination, so results from spectrophotometric measurements of iridescent plumage can be very sensitive to changes in the measurement geometry (e.g. Cuthill et al. 1999; Santos et al. 2007; Maia et al. 2009), which influence both the hue and the chroma of a colour. As we were not able to guarantee exactly the same measurement geometry under fieldwork conditions, we decided to only include speculum brightness as one aspect of colouration in our study. Birds in good body condition could be more tolerant to stressful situations, or both a good body condition and speculum characteristics could indicate good genetic quality. This is supported by Gladbach et al. (2010c), who found that the *H*/*L* ratio (a common measure of stress in birds) is related to body condition in male upland geese, with males in poor body condition showing higher stress measures. The higher body conditions of males with darker specula could also be linked to a condition dependency of the feather moult. Skipping the moult of the primaries for up to three consecutive years has been reported in upland geese before (Summers 1983b), and the gradual impairment of flight feathers might be unimportant to this nonmigratory species. Skipping the moult could have the advantage of saving energy and nutrients, and the colour of the speculum feathers may then be subject to degradation, leading to a paler colour of the older feathers of males in poor body condition. A study sampling feathers from the speculum and spectrophotometric measurements performed under standard laboratory conditions could further highlight the signalling aspect of speculum colouration.

The relationship of male wing colouration to female reproductive investment could be caused by high-quality males in good body condition gaining access to or being able to defend high-quality territories, thereby providing the basis for a high investment in clutch size by their female. However, as the relationship was no longer significant after controlling for hatching date, a study using a larger data set is needed to clarify whether the relationship between clutch volume and male colouration can be verified. While reproductive parameters like egg size seem to be related to the inherent quality of a female in upland geese, clutch size appears to be flexible and to vary according to the current conditions (Gladbach et al. 2010b). Additionally, Gladbach et al. (2010a) found that in upland geese, females with more orange-coloured legs and more red-like head colours had higher clutch and egg volumes than females with paler leg and head colouration, and a more reddish plumage colouration was related to a higher body condition. Thus, in females, these aspects of plumage and integument colouration may be used as quality signals rather than the speculum. As upland geese form long-term pair bonds, a study comparing clutch data, male wing colouration and territory quality in different years is now necessary to understand the relationship of male colouration to female investment.

In summary, we found that, contrary to our expectations, the brightness of the speculum rather than the white plumage was an indicator of condition in male upland geese. In contrast, wing colouration was not related to condition parameters in females. Further studies should now concentrate on other spectrophotometric colour parameters of the iridescent speculum to gain more knowledge about its signalling ability in upland geese as well as in other Anatidae where this special wing characteristic occurs.

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EIGHT

General discussion

GENERAL DISCUSSION

The main goal of this thesis was to identify fitness correlates in wild Upland geese and describe their variation within individuals between different years. In this chapter I summarize and discuss the major findings of this thesis, and suggest some directions for future research.

Investigating the relation of individual characters to fitness often sounds easier than it is, mainly because the actual measurement of fitness is difficult in field studies. Fitness is defined as the ‘contribution made to a population of descendants by an individual relative to the contribution made by others in its present population’ (Begon et al. 1996), so not only the number of offspring during an individuals’ lifetime should be taken into account, but also their fecundity by measuring the number of grandchildren. However, in most ecological studies this is hard to assess and proxies for fitness are used. In this thesis I used egg size, clutch size, hatching date and body condition as proxies for fitness, as all of these form the basis for reproductive output and lifetime reproductive success.

Especially in precocial species like waterfowl female egg investment may play an important role in determining survival prospects of offspring (Amat et al. 2001) and thereby increasing individual fitness. Larger eggs may enhance offspring fitness by (a) increasing survival prospects in the first days after hatching and (b) increase competitive ability through larger offspring size and the possession of more resources to survive adverse conditions (Ankney 1980; Amundsen and Stokland 1990; Magrath 1992; Dawson and Clark 2000; Anderson and Alisauskas 2002; Christians 2002; Goth and Evans 2004; Rutkowska and Cichon 2005).

Table 1 Relation of morphological and metabolic traits to fitness proxies in male and female Upland geese. *Fitness proxies*: ‘0’: no significant effect (i.e. P>0.05), ‘+’: positive relationship (i.e. increase or decrease together), ‘-‘: negative relationship (i.e. one increases while the other decreases). *Repeatability*: √ significant at the P>0.05 level; (√) significant at the P>0.10 level; ‘-‘: not significantly repeatable.

		Body condition	Hatching date	Clutch size	Mean egg volume	Repeatability
Males	Body condition		0	0	0	-
	Faecal GCM	-	0	0	0	√
	H/L	-	0	0	0	-
	Leucocytes	0	0	0	0	(√)
	Plasma protein	0	0	0	0	-
	Plasma triglyceride	0	0	0	0	-
	Plasma carotenoids	0	0	0	0	-
	Wing brightness	0	0	0	0	√
	Speculum brightness	+	0	-	0	(√)
Females	Body condition		-	+	+	√
	Faecal GCM	-	+	0	0	√
	H/L	0	0	0	0	√
	Leucocytes	0	+	-	0	-
	Plasma protein	0	0	+	0	-
	Plasma triglyceride	0	0	0	0	-
	Plasma carotenoids	0	0	0	+	√
	Tarsus colour (more orange)	0	-	+	+	√
	Head colour (more red)	+	0	0	0	-

Additionally early-nesting birds often lay bigger clutches; their offspring grow more rapidly and have a higher chance of survival and recruitment than late-nesting birds (Drent and Daan 1980; Hochachka 1990; Blums et al. 2002; Sockman et al. 2006).

The phenotypic traits studied in this thesis include morphological and metabolic variables. Table 1 provides an overview of the main results of the relation of different traits to proxies for fitness separately for males and females. As an individuals' phenotype is based on both genetic and environmental factors, but selection can only act on the genetic component we used repeatability as a rough estimate for the heritability of a trait. Traits that are both related to a fitness proxy and repeatable are very likely to be affected by selection.

The results differ between the sexes; I therefore will discuss males and females separately.

Males

Most of the phenotypic traits included in our study were not correlated to fitness proxies in males (Tab.1). Only speculum brightness was associated with body condition and clutch size with individuals with darker specula being in a better body condition and females mated to males with a darker speculum laid bigger clutches.

Avian wing displays can signal aggressiveness or willingness to escalate fights (Hurd and Enquist 2001) and white coverts and speculum in the Upland goose may function as 'coverable badges' (Hansen and Rohwer 1986) that are only exposed when birds are highly motivated to fight but hidden when birds are not willing to engage in an aggressive encounter. Differences in male wing colouration may serve as an indicator of status or condition and reduce the need for costly aggressive interactions. The relationship of male wing colouration to female reproductive investment could be caused by high quality males in good body condition gaining access to or being able to defend high quality territories, thereby providing the basis for a high investment into clutch size by their female. While reproductive parameters like egg size seem to be related to the inherent quality of a female in Upland geese; clutch size rather appears to be flexible and vary according to current conditions (Gladbach et al. 2010), which could explain the potential effect of territory quality.

The higher body condition of males with a darker speculum could be linked to a condition dependency of the moult of feathers. Moult skipping of primaries in up to three consecutive years has been reported in Upland geese before (Summers 1983), and the gradual impairment of flight feathers might be unimportant to this non-migratory species. The skipping of moult could have the advantage to save energy and nutrients and the colour of speculum feathers might then be subject to degradation leading to a paler colour of older feathers of males in low body condition. This could also explain why we found speculum brightness to only be marginally significantly repeatable. Testing repeatability depending upon male condition using a larger sample could further highlight the use of speculum colouration as a fitness correlate in male Upland geese.

Females

We found several morphological and metabolic traits to be correlated to different fitness proxies in female Upland geese (Tab.1). Mean egg volume as a measure of individual quality has been discussed before, with phenotypic and genetic factors predisposing some individuals to perform better under a given set of conditions and lay larger eggs (Reid and Boersma 1990; Croxall et al. 1992; Blackmer et al. 2005; Ardia et al. 2006). The positive relation of body condition, plasma carotenoid concentration and orange tarsus colouration to egg size together with their significant repeatability strongly supports their use as a measure of individual quality in female Upland geese. Especially important are the results for female colouration. No study so far investigated the potential of female-specific colouration as a quality signal in birds with conventional sex-roles and only a small number of previous studies reports positive relationships between female-specific ornamentation and aspects of phenotypic quality (Domb and Pagel 2001; Weiss 2006; Weiss et al. 2009). We furthermore report for the first time in a wild bird under natural conditions that a strongly environmentally determined trait signal like carotenoid-based integument colouration is consistent within individuals over time.

Traits related to the timing of breeding and clutch size were not repeatable in most cases (repeatability of faecal GCM was measured within one breeding season, but not between the different study years because of a too low sample size). Both timing of breeding and clutch size have to be viewed in a life-history context. Life-history theory suggests that individuals trade-off the investment of limited resources into current reproduction against self maintenance and survival to reproductive episodes in the future (Stearns 1992). Selection maximises lifetime reproductive output, therefore individual females may decide to breed early or late or lay larger or smaller clutches depending upon both their own current state and environmental conditions. This variation in trait expression is called phenotypic plasticity (West-Eberhard 1989). Selection will favour individuals where the genetic basis enables phenotypic adaptation to current conditions maximising lifetime reproductive success. The missing repeatability of the traits related to timing of breeding and clutch size may reflect this phenotypic plasticity. A study including individuals sampled over a longer period of time, ideally their whole reproductive life-span, could separate phenotypic plasticity from evolved adaptations.

There are two possible explanations for the different patterns we found in our study for fitness correlates in male and female Upland geese:

1. Fitness proxies

The fitness proxies we used in our study could be more likely to detect correlations in females. Especially clutch measures and hatching date are traits that are closely linked to differences in individual quality in females. Both timing of breeding and number and size of eggs are affected by the condition of the female prior to laying (Lack 1968; Ryder 1970; Bengston 1971; Johnsgard 1973; Winkler and Walters 1983). Early-nesting birds often lay bigger clutches; their offspring grow more rapidly and have a higher chance of survival and recruitment than late-nesting birds (Drent and Daan 1980; Hochachka 1990; Blums et al. 2002; Sockman et al. 2006), which results in a decrease in offspring value within a season. High-quality individuals in high-quality territories may be the first to start laying in the breeding season, while low quality individuals in lower quality territories or individuals facing less favourable feeding conditions would lay later (Rowe *et al.* 1994; Sockman *et al.* 2006).

Because of the close connection of these fitness proxies to condition, a relation to morphological and metabolic traits may be easier to discover in females. In males, these proxies are a more indirect measure of reproductive success as they depend on female condition which males cannot influence directly. Males only have the opportunity to choose or gain access to a high quality mate, or provide good feeding conditions by establishing and defending a high quality territory. The sample size used in this study may be too small to detect such indirect effects.

While female quality mainly affects the basis for reproductive success by laying early and large eggs, male quality may be important later in the breeding cycle by providing a high quality territory for offspring growth and defending chicks against predators and during territorial fights. To estimate the effect of male quality on offspring performance it is necessary to measure offspring growth, ideally until fledging, which was logistically not possible in this study.

Additionally, males may increase lifetime reproductive success through extra-pair paternities (EPP). Extra-pair offspring is found in over 90% of all bird species, even in socially monogamous species where on average 11% result from matings outside the social bond (Griffith et al. 2002). A study using molecular tools to determine parentage of offspring, especially in adjacent territories is necessary to gain knowledge on EPP in Upland geese and then link the rate to phenotypic traits in males.

2. Timing of sampling

Another aspect possibly affecting our results is the time when phenotypic traits were measured. We caught adults during the period when they attended their brood (chick age 0-45 days, mean 11±1.4 days). However, traits may show phenotypic plasticity and vary over the breeding cycle.

Repeated sampling of individuals would be necessary to determine the variation of traits over the year; ideally measuring them right at the start of a breeding season before egg laying, during incubation, after hatching, just before fledging of chicks and again during the winter. Again, this was not possible in this study due to logistical limitations.

Where from here?

Clutton-Brock and Sheldon (2010) just pointed out the importance of long-term, individual-based studies of animals in ecology and evolutionary biology. Many of the most important ecological and evolutionary processes affecting the demographic processes controlling population size and the evolutionary processes generating adaptation normally occur over multiple years or decades rather than across hours, weeks or months. Studies of naturally regulated populations that provide data that extend over adequate periods of time are consequently crucial to research in many areas of ecology and evolutionary biology. They identified six advantages that these studies have for the answering of substantial questions. Individual-based studies allow the analysis of age structures, the linkage between different life-history stages, the quantification of social structures, the estimation of lifetime reproductive success, the replication of selection estimates and the linkage between generations.

The study system presented has high potential to be used for such a long-term study. Upland geese have an average expectation of adult life of about five years (Summers and McAdam 1993), although individuals have been found to get up too 16 years in captivity. This moderate life expectancy provides a good basis to monitor the life history of individuals. Upland geese are socially monogamous and highly territorial, returning with the same to the same territory every season, making it easy to find individuals in subsequent years. They are not migratory and winter shedding sites are usually within 5 km of their breeding territory, birds can therefore also be studied during the non-breeding time without difficulty. Additionally, New Island with its area of approximately 2000 ha can be monitored with a manageable amount of time and manpower. Upland geese are big enough to be marked with plastic rings readable from a distance, enabling the collection of observational data and the identification of individuals without the need to capture them. Also the linkage between generations is easily possible as most chicks begin to nest near their natal territory.

The results on fitness correlates in wild Upland geese presented in my thesis may now be used as a starting point for the investigation of complex relationships in ecology and evolutionary biology using long-term monitoring of a population.

SUMMARY

Studies of selection in the wild have continually increased since Darwin introduced his theory 150 years ago. However, despite the large number of studies that can be found for birds, there is a considerable bias towards studies in Passeriformes and species with a socially monogamous mating system with seasonally changing partners. Also, most studies concentrate on morphology and life history, while studies focussing on selection on physiological traits are still missing. As it is dangerous to build the empirical study of natural selection and microevolutionary biology primarily on data from certain species and traits, generalizations and models should be tested across taxa. As far as possible we should determine whether or not different processes are important in organisms with a variety of life histories.

The focus of this thesis is the identification of fitness correlates in a free-ranging population of Upland geese *Chloephaga picta leucoptera* on the Falkland Islands. Upland geese belong to the order Anseriformes, they form stable social monogamous pair bonds over several years and show a strong sexual dimorphism in both size and colouration, a combination underrepresented in the current literature. In this thesis I describe the relationship of several morphological and physiological traits to aspects of fitness in both males and females of this species, and, if applicable, compare them within individuals between years. I thereby aim to identify phenotypic characteristics that are heritable and may have evolved through selection. I address both aspects of selection, describing traits which most likely underlie natural selection (chapters three to five) and others that could have been formed by sexual selection (chapter six and seven). As an individuals' phenotype is based on both genetic and environmental factors, but selection can only act on the genetic component we used repeatability as a rough estimate for the heritability of a trait. Traits that are both related to a fitness proxy and repeatable are very likely to be affected by selection. The phenotypic traits investigated in this thesis include morphological (body mass, body size, tarsus and plumage colouration in females and plumage colouration in males) and metabolic variables (leucocyte counts, faecal corticosterone). I used egg size, clutch size, hatching date and body condition as proxies for fitness, as all of these form the basis for reproductive output and lifetime reproductive success.

In females, egg size was positively related to body condition, plasma carotenoid concentration and orange tarsus colouration, and all of these traits were significantly repeatable, which strongly supports their use as a measure of individual quality. Especially important are the results for female colouration. No study so far investigated the potential of female-specific colouration as a quality signal in birds with conventional sex-roles and only a small number of previous studies reports positive relationships between female-specific ornamentation and aspects of phenotypic quality. I also report for the first time in a wild bird under natural conditions that a strongly environmentally determined trait signal like carotenoid-based integument colouration is consistent within individuals over time.

Traits related to the timing of breeding and clutch size were not repeatable in most cases (repeatable: body condition, tarsus colouration; not repeatable: leucocyte counts, corticosterone). Both timing of breeding and clutch size have to be viewed in a life-history context and the trade-off between current and future reproduction. Selection maximises lifetime reproductive output, therefore individual females may adapt their timing of breeding and clutch size depending on both their own current state and environmental conditions; a phenomenon called phenotypic plasticity. The missing repeatability of the traits related to timing of breeding and clutch size may reflect this plasticity.

In males, most of the phenotypic traits included in our study were not correlated to fitness proxies. Only speculum brightness was associated with body condition and clutch size with individuals with darker specula being in a better body condition and females mated to males with a darker speculum laid bigger clutches.

The different patterns I report in this thesis for fitness correlates in males and females may be caused by the fitness proxies I used. Because of the close connection of egg size, clutch size and timing of breeding to condition, a relation to morphological and

metabolic traits may be easier to discover in females. In males, these proxies are a more indirect measure of reproductive success as they depend on female condition which males cannot influence directly. Males only have the opportunity to choose or gain access to a high quality mate, or provide good feeding conditions by establishing and defending a high quality territory. While female quality mainly affects the basis for reproductive success by laying early and large eggs, male quality may be important later in the breeding cycle by providing a high quality territory for offspring growth and defending chicks against predators and during territorial fights. Additionally, males may increase lifetime reproductive success through extra-pair paternities. Alternatively, timing of sampling could have affected the results, as traits may show phenotypic plasticity and vary over the breeding cycle.

The results on fitness correlates in wild Upland geese presented in my thesis contribute to the understanding of the selective forces shaping a species' current appearance and distribution in a non-passerine system. The further study of fitness correlates in this species with a long-term pair bond may provide valuable insight into the effects of mate quality on lifetime reproductive success of both pair partners. The strong sexual dimorphism in both size and colouration may be used to study the stability of sexual signals over the time of the pair bond. This study system has a high potential to be used for the investigation of complex relationships in ecology and evolutionary biology using long-term monitoring of a population.

ZUSAMMENFASSUNG

Die Anzahl von Studien zu Selektionsvorgängen in der freien Natur hat kontinuierlich zugenommen, seitdem Darwin seine Evolutionstheorie vor 150 Jahren vorgestellt hat. Trotz der großen Anzahl von Studien an Vögeln gibt es jedoch eine starke Tendenz zu Untersuchungen an Passeriformes und an Arten mit einem sozial monogamen Paarungssystem und jährlich wechselnden Partnern. Weiterhin konzentrieren sich die meisten Studien auf die Selektion von morphologischen oder life history Merkmalen, wohingegen Studien zu physiologischen weitgehend fehlen. Da es jedoch gefährlich sein kann, die empirischen Untersuchungen zu natürlicher Selektion und mikroevolutionärer Biologie allein auf Daten von wenigen Arten und Merkmalen zu stützen, sollten Generalisierungen und Modelle bei verschiedensten Taxa getestet werden, um festzustellen welche Rolle bestimmte Prozesse bei Organismen mit unterschiedlichsten Lebenszyklusstrategien spielen.

Der Fokus meiner Arbeit lag auf der Identifikation von individuellen Fitness Korrelaten bei einer frei lebenden Population von Magellangänsen *Chloephaga picta leucoptera* auf den Falklandinseln. Magellangänse gehören zur Ordnung der Anseriformes, sie bilden über mehrere Jahre stabile, sozial monogame Paarbindungen und zeigen einen auffallenden Sexualdimorphismus in sowohl Größe als auch Färbung. Diese Kombination von Merkmalen ist in der derzeitigen Literatur zu Selektion in Wildpopulationen deutlich unterrepräsentiert. In dieser Arbeit beschreibe ich das Verhältnis mehrerer morphologischer und physiologischer Merkmale zu Fitnessaspekten bei sowohl Männchen als auch Weibchen, und vergleiche wenn möglich die Entwicklung dieser Merkmale über mehrere Jahre innerhalb von Individuen. Dadurch beabsichtige ich phänotypische Eigenschaften zu identifizieren die vererbbar sind und damit durch die Wirkung von Selektion entstanden sein können. Ich befasse mich mit beiden Aspekten der Selektion und beschreibe sowohl Merkmale, die höchstwahrscheinlich natürlicher Selektion unterliegen (Kapitel drei bis fünf) als auch mit solchen, die durch sexuelle Selektion entstanden sein könnten (Kapitel sechs und sieben).

Da der Phänotyp eines Individuums auf genetischen und Umweltfaktoren beruht, Selektion aber nur den vererbbaren genetischen Anteil beeinflussen kann, nutze ich Wiederholbarkeit (repeatability) als ein grobes Maß für die Vererbbarkeit (heritability) eines Merkmals. Merkmale, die sowohl mit einem Fitnessmaß als auch innerhalb von Individuen wiederholbar sind, werden höchstwahrscheinlich durch Selektion beeinflusst. Die phenotypischen Merkmale, die ich in dieser Arbeit untersuche beinhalten morphologische (Körpermasse, Körpergröße, Tarsus- und Gefiederfärbung bei Weibchen und Gefiederfärbung bei Männchen) und physiologische (Leukozyten, Stresshormone) Variablen. Ich habe Eigröße, Gelegegröße, Schlupfdatum und Körperkondition als Fitnessmaße verwendet, da diese die Basis für Reproduktionserfolg darstellen.

Bei Weibchen hing die Eigröße positiv mit der Körperkondition, der Carotenoidkonzentration im Blutplasma und der Intensität der orange-gelben Tarsusfärbung zusammen. All diese Merkmale waren außerdem wiederholbar, was ihre Verwendung als individuelle Qualitätsmerkmale unterstützt. Besonders wichtig sind meine Ergebnisse zur weiblichen Färbung. Bisher hat keine Studie die potentielle Rolle von Weibchen-spezifischer Färbung als Qualitätssignal bei einer Vogelart mit konventionellen Geschlechterrollen untersucht, und nur eine kleine Anzahl von Studien berichtet von einem positiven Zusammenhang zwischen weiblichen Ornamenten und Aspekten phänotypischer Qualität. Weiterhin zeige ich das erste Mal für eine freilebende Art, dass ein stark umweltbestimmtes Merkmale wie carotenoidbasierte Integumentfärbung innerhalb von Individuen über mehrere Jahre stabil bleibt.

Merkmale die mit dem Brutzeitpunkt und der Gelegegröße in Zusammenhang standen, waren nur zum Teil wiederholbar (wiederholbar: Körperkondition, Tarsusfärbung; nicht wiederholbar: Leukozytenzahlen, Stresshormone). Sowohl der Brutzeitpunkt als auch die Gelegegröße müssen dabei unter dem Gesichtspunkt der Lebenslauftheorie und dem

Kompromiss zwischen aktueller und zukünftiger Reproduktion betrachtet werden. Selektion wirkt in Richtung der Maximierung des lebenslangen Reproduktionserfolgs, weshalb Weibchen dahingehend selektiert werden, den Zeitpunkt des Brutbeginns und die Gelegegröße sowohl ihrem eigenen momentanen Zustand als auch den herrschenden Umweltbedingungen anzupassen, ein Phänomen das man phänotypische Plastizität nennt. Die fehlende Wiederholbarkeit von Merkmalen die in einem Zusammenhang mit Brutzeitpunkt und Gelegegröße stehen könnte diese phänotypische Plastizität widerspiegeln.

Bei Männchen hingen die meisten der untersuchten phänotypischen Merkmale nicht mit einem der in dieser Studie verwendeten Fitnessmaß zusammen. Nur die Helligkeit des Flügelspiegels war positiv mit der männlichen Körperkondition korreliert und der Gelegegröße ihrer Partnerinnen.

Eine mögliche Ursache für die unterschiedlichen Ergebnisse die ich bei Magellangansmännchen und -weibchen in meiner Arbeit gefunden habe könnte die Wahl der Fitnessmaße sein. Aufgrund der engen Verknüpfung von Eigröße, Gelegegröße und Brutzeitpunkt und weiblicher Körperkondition, könnte ein Zusammenhang zwischen diesen Variablen und morphologischen und physiologischen Merkmalen einfacher bei Weibchen zu finden sein. Bei Männchen sind diese Fitnessmaße indirekt mit dem Reproduktionserfolg verknüpft, da sie auf der Kondition ihrer Partnerinnen beruhen, auf die sie keinen direkten Einfluss haben. Für Männchen besteht nur die Möglichkeit der Wahl einer Partnerin in guter Kondition oder die Bereitstellung von guten Futterbedingungen durch die Etablierung und Verteidigung einen qualitativ hochwertigen Territoriums. Während die Qualität des Weibchens durch einen frühen Legebeginn und ein großes Gelege mit hochvolumigen Eiern hauptsächlich im Zusammenhang mit der Basis des Reproduktionserfolges steht, könnte die Qualität des Männchens später im Brutzyklus eine größere Rolle spielen, z.B. durch die Bereitstellung eines guten Territoriums und die Verteidigung des Nachwuchses gegen Prädatoren und während Territorialkämpfen. Weiterhin können Männchen ihren lebenslangen Reproduktionserfolg durch die Zeugung von Nachwuchs außerhalb des sozialen Paarbundes erhöhen. Auch die Wahl des Zeitpunktes der Datenaufnahme könnte die Ergebnisse beeinflusst haben, da vor allem phänotypisch plastische Merkmale im Laufe des Reproduktionszyklus variieren können. Eine weiterführende Studie könnte sich mit diesen Punkten befassen.

Meine Ergebnisse zu Fitnesskorrelaten bei frei lebenden Magellangänsen tragen zum Verständnis der Selektionskräfte bei einer nicht-passeriformen Art bei. Weitere Untersuchungen an dieser Art mit Langzeitpaarbindung können wertvolle Ergebnisse zum Einfluss der Partnerqualität auf den lebenslangen Reproduktionserfolg beider Paarpartner liefern. Ferner eignet sich der starke Sexualdimorphismus bei Magellangänsen dazu, die Stabilität von sexuellen Signalen während der Dauer der Paarbindung zu untersuchen. Das hier vorgestellte Studiensystem eignet sich hervorragend zur Untersuchung komplexer ökologischer und evolutionsbiologischer Fragestellungen, die oft nur durch das Langzeitmonitoring einer Population frei lebender Tiere beantwortet werden können.

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CURRICULUM VITAE

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1 *Education*

since 10/2007	Max Planck Institute for Ornithology, Vogelwarte Radolfzell Doctoral thesis „Individual fitness correlates in consecutive years of pair bond in Upland Geese" Supervisors: Prof. Dr. Bart Kempenears, Dr. Petra Quillfeldt
1999-2005	Studies in Biology Friedrich-Schiller-University, Jena, Germany (Ecology, Botany, Tropical ecology) Diploma thesis, Institute of Ecology „Begging behaviour of Wilson's storm-petrel (*Oceanites oceanicus*) and its effect on parental investment." Supervisors: Prof. Dr. Stefan Halle, Dr. Hans-Ulrich Peter.
1992-1999	Gymnasium Zschopau
1987-1992	Martin-Andersen-Nexö OS Zschopau, Primary school

2 *Grants and Awards*

Since 10/2007	Scholarship of the Bayerischen Eliteförderung
2009	Grant from the German Academic Exchange Service (DAAD) to participate in the 7th European Ornithologists Union Conference, Zurich, Switzerland
2009	Grant of the SYNTHESYS Project (ES-TAF 5680) for the molecular determination of blood parasites in collaboration with the Museo Nacional de Ciencias Naturales Madrid
2008	DO-G (German Ornithologists' Society) Research Grant for field assistant
2008	Grant from the German Academic Exchange Service (DAAD) for increased costs of living on the Falkland Islands (DAAD-Aufstockungsstipendium)
2007	DO-G (German Ornithologists' Society) Research Grant
2007	Grant from the German Academic Exchange Service (DAAD) for increased costs of living on the Falkland Islands (DAAD-Aufstockungsstipendium)

2006	Travel grant of the „Arthur-von-Gwinner-Stiftung for scientific expeditions” for a preliminary project on Upland geese in the New Island Nature Reserve, Falkland Islands.
2005	Young Referee Award at the 138th DO-G Conference, Stuttgart, Germany.
2004	Best Poster Award, XXVIII SCAR (Scientific Committee for Antarctic Research) Open Science Conference, Bremen, Deutschland.
1999	Winner of the “Wissenschaftsschreiber-Wettbewerb” of the FSU Jena (Contest on scientific writing). Article about the effects of caffeine (A. Kahles (2000): Ich brauch erst mal ‘nen Kaffee! Uni-Journal Jena, 04/00).

3 Publications

Peer-reviewed journals

Gladbach, A., Gladbach, D. J., Kuchar A., Möstl, E. & Quillfeldt, P. (2011): Can faecal glucocorticoid metabolites be used to monitor body condition in wild Upland geese *Chloephaga picta leucoptera?* Behavioral Ecology and Sociobiology (DOI: 10.1007/s00265-011-1169-3).

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Quillfeldt, P., Martínez, J., Ludynia, K., **Gladbach, A.**, Masello, J.F., Hennicke, J., Riou, S., & Merino, S. (2010): Hemosporidian blood parasites in seabirds – a comparative genetic study of species from Antarctic to tropical habitats. Naturwissenschaften, 97(9):809-817 (DOI 10.1007/s00114-010-0698-3).

Gladbach, A., Gladbach, D. J., Kempenaers, B. & Quillfeldt, P. (2010): Female-specific colouration, carotenoids and reproductive investment in a dichromatic species, the Upland goose *Chloephaga picta leucoptera*. Behavioral Ecology and Sociobiology, 64(11), 1779–1789 (DOI 10.1007/s00265-010-0990-4).

Gladbach, A., Gladbach, D. J. & Quillfeldt, P. (2010): Seasonal clutch size decline and individual variation in the timing of breeding are related to female body condition in a non-migratory species, the Upland goose *Chloephaga picta leucoptera*. Journal of Ornithology, 151, 817-825 (DOI: 10.1007/s10336-010-0518-8).

Gladbach, A., Gladbach, D. J. & Quillfeldt, P. (2010): Variations in leucocyte profiles and plasma biochemistry are related to different aspects of parental investment in male and female Upland geese *Chloephaga picta leucoptera*. Comparative Biochemistry and Physiology - Part A, 156, 269-277 (DOI: 10.1016/j.cbpa.2010.02.012).

Gladbach, A., Braun, C., Nordt, A., Peter, H.-U. & Quillfeldt, P. (2009): Estimation of feeding frequencies by periodic weighing of chicks: evaluation by video-observation of burrow-nesting Wilson’s Storm-Petrels *Oceanites oceanicus*. Emu, 109, 316-320 (DOI: 10.1071/MU09043).

Gladbach, A., Braun, C., Nordt, A., Peter, H.-U. & Quillfeldt, P. (2009): Chick provisioning and nest attendance of male and female Wilson’s storm-petrel *Oceanites oceanicus*. Polar Biology, 32, 1315-1321 (DOI: 10.1007/s00300-009-0628-z).

Gladbach, A., Büßer, C., Mundry, R. & Quillfeldt, P. (2009): Acoustic parameters of begging calls indicate chick body condition in Wilson’s storm-petrel. Journal of Ethology, 27, 267-274 (DOI: 10.1007/s10164-008-0115-y)

Quillfeldt, P., Schenk, I., McGill, R.A.R., Strange, I.J., Masello, J.F., **Gladbach, A.**, Roesch, V. & Furness, R.W. (2008): Introduced mammals coexist with seabirds at New Island, Falkland Islands: Abundance, habitat preferences and stable isotope analysis of diet. Polar Biology, 31, 333-349 (DOI: 10.1007/s00300-007-0363-2).

Büßer, C., Hahn, S., **Gladbach, A.**, Lorenz, S., Nordt, A., Quillfeldt, P., Schmoll, T. & Peter, H.-U. (2008): A decade of fundamental ecological research on storm-petrels at the Tres Hermanos colony, Potter Peninsula, King George Island. Reports on Polar Research, 571, 168-175.

Gladbach, A., McGill R.A.R. & Quillfeldt, P. (2007): Foraging areas of Wilson's storm-petrel *Oceanites oceanicus* in the breeding and inter-breeding period determined by stable isotope analysis. Polar Biology, 30, 1005-1012 (DOI: 10.1007/s00300-007-0258-2).

Büßer, C., **Kahles, A.** & Quillfeldt, P. (2004): Breeding success and chick provisioning in Wilson's storm-petrels *Oceanites oceanicus* over seven years: frequent failures due to food shortage and entombment. Polar Biology, 27, 613-622 (DOI: 10.1007/s00300-004-0627-z).

Conference publications

Gladbach, A., Gladbach, D.J., Quillfeldt, P. (2009). Tarsus colouration as a signal of individual quality in Upland Geese (*Chloephaga picta leucoptera*). Talk, 7th EOU conference, Zürich, Switzerland.

Gladbach, A., Gladbach, D.J., Quillfeldt, P. (2008). „Zuviel der Sorge tut nicht gut, macht bleich und dürr und dünnt das Blut": Untersuchung zum Stresslevel von Magellangänsen anhand hämatologischer Parameter. Talk, 141. Tagung der DO-G, Bremen, Germany.

Quillfeldt, P., McGill, R.A.R., Masello, J.F., Weiss, F., **Gladbach, A.**, Furness, R.W. (2008). Changing places? Contrary winter movements of small seabirds from both sides of the Drake Passage. IsoEcol VI, Honolulu, Hawaii.

Gladbach, A., Büßer, C., Peter, H-U., Quillfeldt, P. (2005). Sind Buntfußsturmschwalben (*Oceanites oceanicus*) ehrliche Bettler? Talk, 138. Tagung der DO-G, Stuttgart, Germany. Young Referee Award.

Gladbach, A., Büßer, C., Peter, H-U., Quillfeldt, P. (2005) Wilson's storm-petrels (*Oceanites oceanicus*) as a model system for the study of parent-offspring interactions. Poster, 22. Internationale Polartagung Jena, Germany.

Büßer, C., Quillfeldt, P., Hahn, S., Schmoll, T., **Gladbach, A.**, Nordt, A., Peter, H.-U. (2005) Long-term research on Wilson's and Black-bellied storm-petrels on King George Island, South Shetland Islands. Talk, 22. Internationale Polartagung Jena.

Peter, H.-U., **Gladbach, A.**, Janicke, T., Pfeiffer, S., Ritz, M. (2005)): Unter Skuas und Sturmschwalben - 20 Jahre Jenaer ornithologische Forschung in der Antarktis. Vortrag, 43. Jahresversammlung und Sächsische Ornithologentagung des Vereins Sächsischer Ornithologen e.V., Hoyerswerda, Germany.

Kahles, A., Büßer, C., Mundry, R. & Quillfeldt, P. (2004) Begging calls of Wilson's storm-petrel and parental investment. Poster, 3rd International Albatros and Petrel Conference, Montevideo, Uruguay.

Kahles, A., Büßer, C., Peter, H-U., Quillfeldt, P.(2004) Bettelverhalten von Buntfußsturmschwalben und elterliches Investment. Poster, 137. Tagung der DO-G, Kiel.

Kahles, A., Büßer, C., Peter, H.-H., Mundry, R. & Quillfeldt, P. (2004) Begging calls of Wilson's storm-petrel and feeding behaviour of adults. Poster, XXVIII SCAR (Scientific Committee for Antarctic Research) Open Science Conference, Bremen. "Best Poster" Award.

Peter, H.-U., Büßer, C., Chupin, I., Janicke, T., **Kahles, A.**, Pfeiffer, S., Ritz, M., Wang, Z. & Welcker, J. (2003): Mortality of Skua and Wilsons Storm Petrel chicks in the Antarctic. 4th EOU conference, Chemnitz, Germany.

Peter, H.-U., Büßer, C., Chupin, I., Janicke, T., **Kahles, A.**, Pfeiffer, S., Prieto. I., Ritz, M.,Wang, Z.& Welcker, J. (2003): Mortalität von Skua- und Sturmschwalben-Küken in der Antarktis. 21. Internationale Polartagung, Kiel, Germany.

Referee activities

Behavioral Ecology & Sociobiology
Polar Biology
Journal of Ornithology
The Auk
Marine Ecology Progress Series
Waterbirds
Marine Biology

Ehrenwörtliche Versicherung

Ich versichere hiermit ehrenwörtlich, dass die vorgelegte Dissertation von mir selbständig und ohne unerlaubte Hilfe angefertigt ist.

München, den .... ……………......................................
Anja Gladbach

Erklärung

Hiermit erkläre ich, dass die Dissertation nicht ganz oder in wesentlichen Teilen einer anderen Prüfungskommission vorgelegt worden ist und dass ich mich anderweitig einer Doktorprüfung ohne Erfolg nicht unterzogen habe.

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Anja Gladbach