

Individual fitness correlates in the Black-tailed Godwit

Julia Schroeder



rijksuniversiteit
 groningen



The research presented in this thesis was carried out at the Animal Ecology Group, which is part of the Centre for Ecological and Evolutionary Studies (CEES) of the University of Groningen (RuG). The research was supported by a start-up grant from the University of Groningen to the professorship in animal ecology of Theunis Piersma. Production of this thesis was partly funded by the University of Groningen and the Centre for Ecological and Evolutionary Studies.

Lay-out and figures: Dick Visser

Photo cover: Julia Schroeder

Printed by: Drukkerij van Denderen BV, Groningen

ISBN: 978-90-367-4157-6

ISBN: 978-90-367-4158-3 (electronic version)

RIJKSUNIVERSITEIT GRONINGEN

Individual fitness correlates in the Black-tailed Godwit

Proefschrift

ter verkrijging van het doctoraat in de
Wiskunde en Natuurwetenschappen
aan de Rijksuniversiteit Groningen
op gezag van de
Rector Magnificus, dr. F. Zwarts,
in het openbaar te verdedigen op
maandag 11 januari 2010
om 13:15 uur

door

Julia Schröder

geboren op 25 mei 1976
te Ibbenbüren, Duitsland

Promotor: Prof. dr. T. Piersma

Copromotor: Dr. C. Both

Beoordelingscommissie: Prof. dr. F. Bairlein
Prof. dr. B. Kempenaers
Prof. dr. A. Roulin

Contents

| | | |
|---------------|---|-----|
| Chapter One | Introduction | 7 |
| Box A | Black-tailed godwits | 15 |
| Chapter Two | Space use by black-tailed godwits <i>Limosa limosa limosa</i> during settlement at a previous or a new nest location | 21 |
| Box B | Early breeding | 31 |
| Chapter Three | Caught between climate change and agricultural intensification: a grassland shorebird fails to adjust breeding date but does alter reproductive investment | 37 |
| Chapter Four | Sexual dimorphism in plumage and size in black-tailed godwits <i>Limosa limosa limosa</i> | 53 |
| Chapter Five | A possible case of contemporary selection leading to a decrease in sexual plumage dimorphism in a grassland-breeding shorebird | 69 |
| Chapter Six | Intrasexual competition predicts a positive relation between plumage ornamentation and female reproductive investment, but not with timing of breeding in the Icelandic black-tailed godwit | 89 |
| Chapter Seven | Degree of melanin plumage coloration in a grassland-breeding shorebird: interpretable correlations with nest density | 99 |
| Box C | Diet | 109 |
| Chapter Eight | Linking intronic polymorphism on the CHD1-Z gene with fitness correlates in black-tailed godwits | 117 |
| Chapter Nine | Seasonal carry-over effects: feather isotope signatures of diet during spring stopover correlate with female arrival at breeding site, body mass and egg volume in black-tailed godwits | 131 |
| Chapter Ten | Synthesis | 143 |
| | References | 169 |
| | Summaries | 185 |
| | Acknowledgements | 201 |
| | Author addresses | 204 |

One

Introduction

Natural selection

VARIATION BETWEEN INDIVIDUALS arises due to three factors: mutation, sexual recombination and gene flow between populations. For natural selection to occur, heritable variability must occur in the focal trait (a distinct variant of a phenotypic character), and the phenotypes must vary in fitness. These form the basis for natural selection (Fisher, 1958). Thus traits that vary between individuals and are correlated with fitness are most likely interesting to study, because all evolution begins with adaptive changes of phenotypes, and that is observable in nature.

Ecology

NATURAL SELECTION is the most important process that leads to adaptive changes in phenotypes. Favorable and adaptive traits that increase the fitness of the bearer are selected for, while maladaptive traits are selected against (Fisher, 1958). It is often implied that this process happened long time ago. However, such would mean that all trait variation by now be fixed, and that organisms would be perfectly adapted to their environment - and this is of course not true. One of the reasons why is that environmental conditions are usually far from stable. The famous title of one of Dobzhansky's essays "Nothing in biology makes sense without evolution" (Dobzhansky, 1973) was augmented to "Nothing in evolution makes sense but in the view of ecology" by the authors of a well-known ecology textbook (Townsend, Harper & Begon, 2006), a very important remark for the study of natural history of organisms in changing environments. The, most often stochastic environment is ultimately the driver of changing selection pressures. It can at times favor a certain phenotype, while at other times, a different one. This is why a perfectly optimized organism will never exist: it will only be adapted to its environment to a certain degree, because a static environment is very rare in nature (Mayr, 1982). These changes of the environment can be of abiotic nature, but it also happens that organisms influence each other. And again, differences between individuals make them differently well adapted to different situations, preserving variation (Mayr, 1982). Some traits may be phenotypically variable, but not heritable and hence cannot be optimized by selection forces. We may observe a change in mean phenotype, while not one in genotype. However, in such cases, evolution selects for organisms whose phenotypic plasticity is able to express a trait in a way that it leads to the owner gaining a higher fitness than the population average (West-Eberhard, 1989). Under directionally changing environmental conditions, selection pressures will lead to directional change of heritable traits and evolution will happen (Fisher, 1958).

Changing environment and adaptation

WHEN STUDYING A POPULATION of organisms it is often assumed that all alleles are fixated (contemporarily), and explanations for fitness correlations are usually sought for in the pre-historical past of the species only. The progress of evolution is of course ongoing. There is much discussion on the rate and the shape of the temporary distribution of evolution (Eldredge & Gould, 1972; Dennett, 1995; Dawkins, 1996). Although probably unlikely, we can never exclude the possibility of evolution happening right now while we observe it. This is even more likely as we currently experience severe, most often man-made, environmental changes like global climate change, large-scale land use changes, changes of ecosystems networks due to unsustainable management and the like. We are currently living during one of the time periods during the evolution of life where environmental conditions for many, if not most species change very fast. The current wave of species extinction is a result of such (human inflicted) changes. Currently, a large body of literature on microevolution, or contemporary evolution, is accumulating (see Carroll *et al.*, 2007 for an overview). In addition, many contemporary studies focus on the responses of organisms to directional global changes, most often climate change (for instance Crick & Sparks, 1999; Both & Visser, 2005a; Robinson *et al.*, 2008; Both *et al.*, 2009). These responses are often phenotypically plastic, but it is also likely that with ongoing climate warming these responses will lead to a change of these traits over time. Thus the assumption that the state we are currently observing is a stable one is often made but a rather ad-hoc and imprecise one.

This is why it is interesting to look at individual differences. Individual differences that correlate with fitness can tell us about past and current selection pressures. It might be that most of the traits we observe are evolutionary stable, and few indeed may be directly under selection. Studying individual fitness correlates in a comparative manner can teach us much about the species' phenotypic potential with respect to abiotic changes. Also, it can teach us about the limits of phenotypic flexibility. It can further our understanding of how evolution shapes the way individuals behave and look, and maybe even give us some insight in what might happen for a given future environmental scenario.

Global change and Black-tailed Godwits

THE WORLD WE LIVE IN is constantly changing, currently at a high pace, where environments are severely transformed within a few decades only. We humans have changed and affected virtually all ecological zones and habitats inhabited by other organisms on this globe. Forests, steppes and even sea floor have been turned to agricultural fields, rivers have been dammed up and regulated, coral reefs got bleached, seas polluted. We removed mountaintops, created islands where there were none before; we dug mines, irrigated deserts and drained swamps. Human induced climate

change affects ecosystems from the Himalayas to those on the deepest ocean floor. There are not many, if any, areas on this earth that we have not modified in one way or the other. All species are affected by these changes, and the way they cope with those differs widely. Quite some species arranged themselves to live of and breed in anthropogenically-transformed landscape. A small selection of those species are the meadowbirds, among them the black-tailed godwit (*Limosa limosa limosa*), which is the focal species in this thesis.

In The Netherlands, black-tailed godwits originally bred in peat bogs and fens (Haverschmidt, 1963, Beintema *et al.*, 1995). During the first half of the last century, many of those areas were remodeled for agricultural purposes (Bijlsma *et al.*, 2001). The black-tailed godwit, similar to other meadowbirds like Redshank and Northern lapwing, moved with the change and started breeding on cultivated land (Haverschmidt, 1963, Beintema *et al.*, 1995). This was apparently beneficial: the new hay meadows provided food in high abundance and availability; the more organic dung applied, the better (Beintema *et al.*, 1985; Bijlsma *et al.*, 2001). This increase in food availability is thought to have triggered the golden era of the meadowbirds – a handful of bird species utilizing human-made agricultural hay land for their benefit that bred in rapidly increasing numbers on the Dutch grasslands (Thijssen, 1904; Beintema *et al.*, 1985; Beintema *et al.*, 1995; Bijlsma *et al.*, 2001). Meadowbirds soon became very popular among farmers, nature lovers and birders alike. They became inseparably associated with the Dutch agricultural countryside, the Black-tailed godwit leading in popularity as the “King of the Meadowbirds” (Thijssen, 1904). By 1960, godwits were mainly found on grassland, and barely, if ever, in peat bogs and fens (Haverschmidt, 1963).

However, when the amount of artificial fertilizers used increased the landscape was transformed again. Agricultural intensification, namely water drainage, high nitrogen loads and new, fast-growing plant species allowed more than one harvests, with increasing yields per year (Bijlsma *et al.*, 2001). This led to multiple, advancing mowing dates on the now intensively managed agricultural grassland, with low plant and insect diversity. This did not benefit the meadowbirds: clutch losses due to large agricultural machinery, insufficient food availability for chicks, increased predation due to reduced cover after mowing and habitat loss in general led to a rapid decrease of the numbers of breeding meadowbirds, especially Black-tailed godwits (Beintema *et al.*, 1995; Bijlsma *et al.*, 2001; Schekkerman & Beintema, 2007; Schekkerman *et al.*, 2008; Theunissen *et al.*, 2008; Schekkerman *et al.*, 2009). Since the late 1970ies, the Dutch godwit population decreased by 5% annually, and despite the unchanged popularity of godwits as the kings of the meadows and despite major conservation actions and financial effort taken we have no reason to believe that the decrease will stop anytime soon (Thorup, 2006; Gill *et al.*, 2008; Schekkerman *et al.*, 2008).

For these reasons it is interesting to look at individual fitness correlates in black-tailed godwits and link them with changes over the time when environmental changes occurred.

Outline

IN THIS WORK we search for and discuss fitness correlates in the black-tailed godwit (*Limosa limosa*). My colleagues and me study patterns interesting to most integrative evolutionary ecologists, discuss fitness correlates and their suggestive power of how selection may act and may have acted on black-tailed godwits. However, most of the chapters are also interesting from an applied point of view. A more detailed knowledge of fitness correlates and selection forces equips us with knowledge on where to place the levers which might enable us to change population development towards a more positive direction. I hope that this thesis can add a tiny piece to the large picture.

In the box: **‘Black-tailed Godwits’** I give an overview about the three subspecies of the black-tailed godwit, their distribution, historic and recent population development and possible causes for differences. In **Chapter two** (‘Space use by black-tailed godwits’), we describe the behavior of godwits after they arrive in late winter on their breeding grounds in The Netherlands. We show that birds return to their old breeding site of previous years and stay in the close vicinity of that location for a few days to weeks. Birds that later breed at their old nesting site, continue to stay very close to that location for several weeks, while birds that end up breeding somewhere else gradually move further away, suggesting some kind of prospecting behavior. This is new insight, as before it was thought that most birds assess the quality by means of reproductive success of their previous breeding site during the previous year, and base decisions on where to breed in future on this information. We now show that godwits also use information of the new season for the decision of where to breed. We also show that territories get established soon after arrival and that birds spend most of their time in a very small area close to the future nest site. This has consequences, for instance, about the role of the exact spatial distribution of parameter that make up habitat quality. In this Chapter, we also introduce the core study site, the Workumerwaard.

In the box **‘Early breeding’** we show that early egg laying may be beneficial for godwits, even if breeding in a nature reserve with a late mowing regime. In **Chapter three** (‘Reproductive timing and investment’) we show that penalties for late breeding increased over the last three centuries, very likely due to human interferences. Surprisingly, we find no change in laying date despite advancement in timing of breeding in the first six decades of the 20th century, - which suggests that phenotypical flexible timing of breeding may have reached a limit. We show that godwits nowadays invest more into early clutches, especially so in a year with a preceding warm winter than they would do 30 years ago. We also show that egg volume, chick mass and timing of breeding are determinants for chick survival, and therefore crucial for recruitment rates.

In **Chapter four** (‘Sexual dimorphism in black-tailed godwits’) we define and refine measurements of body dimensions and, most important, plumage assessment. We quantify sexual dimorphism on plumage and body dimensions, and we infer that some plumage traits are sexually selected traits. Additionally, we reevaluate molecular sexing methods and propose a solution for one common molecular sexing error. We

here first introduce the idea that variation on the CHD1-Z gene may covary with fitness correlates in the godwits, suggesting that there may be a genetic basis for different plumage phenotypes.

In **Chapter five** ('Gradual decrease of sexual plumage dimorphism') we use the formerly defined plumage scores and explore whether they correlate with reproductive success in our contemporary breeding godwit population. We find that they do correlate, however, in a most unexpected way: more pale males are the better ones. This leads us to hypothesize that selection should favor pale males. Indeed it turns out that male godwits of the nominate race got paler over the course of the last 164 years, leading the sexual plumage dimorphism to decrease. Nowadays, males that look more like females are more successful as opposed to the more colorful males. We suggest that decreased competition for territories may have led to relaxed selection for brightly ornamented males, and that this may have favored less ornamented males.

In **Chapter six** ('Male plumage and female reproductive investment in the Icelandic black-tailed godwit'), we set out and conduct a similar contemporary analysis as in Chapter five, but for godwits of the Icelandic subspecies, that have a different natural history. In this species, the population size increases currently and competition for nesting sites on high quality breeding grounds is presumably high, which hypothetically should lead to a positive relationship between male plumage ornamentation and reproductive success. We show that more ornamented males are indeed paired with females that invest more into reproduction than less ornamented males.

In **Chapter seven** ('Correlations with density'), we show that breeding in higher densities may be advantageous, and we speculate whether having a colorful plumage may prevent godwits from breeding in high densities.

In **Chapter eight** ('Link between an intronic length polymorphism and fitness correlates') we repeat the exercise of Chapter four, and test a larger part of the population for covariation of the intronic variation on CHD1-Z with fitness correlates. Thus godwit plumage has indeed a genetic basis. We show that this marker is present in high quality birds breeding in high quality breeding area. Further, there is evidence for population structure in relation to breeding habitat quality because birds with the high quality genetic marker are only found in high quality breeding habitat, but never outside. We find that this polymorphism is not a new and recent mutation, as it was already present in a godwit that died in 1929 and ended up as a museum specimen in the Copenhagen museum.

In the box on **'Effects of Diet'**, we describe results of an experiment on captive godwits during spring staging. Godwits feeding on rice gained less weight, but developed a more colorful breeding plumage than godwits feeding on fly larvae.

In **Chapter nine** ('Seasonal carry over effects') we tried to disentangle different foraging and staging strategies during spring migration. We link isotopic signatures of breeding feathers made on the spring staging areas with fitness correlates. We find a relationship between female arrival on the breeding site, body mass and isotope signature, and also of egg volume and isotope signature, but we fail to make a clear statement on whether there is a link with a certain location or a certain type of food,

mainly because isotopic signatures of different food items from different locations are not clearly distinguishable.

In **Chapter ten** I try to integrate our findings.

Box A

Black-tailed godwits

Julia Schroeder

Habitat and breeding range

BLACK-TAILED GODWITS (*Limosa limosa*) are migratory shorebirds and breed in temperate areas. The main breeding habitat is wet grassland (Beintema, Moedt & Ellinger, 1995), but godwits also breed in swamps, bogs and on wet depressions in the steppe (Piersma, van Gils & Wiersma, 1996). The birds spend the winter in wetlands, marshes, estuaries, rice fields and mudflats (Beintema et al., 1995). Since fifty years, rice fields are a major additional wintering habitat (Piersma et al., 1996; Zwarts et al., 2009). The breeding range of black-tailed godwits reaches from Iceland to East-Siberia, and the wintering ranges from Ireland, Great Britain, Southern Europe, Africa to the Middle East, India, and South-east Asia to Australia (Figure A1; Piersma et al., 1996).

Subspecies

THERE ARE THREE GENETICALLY DISTINCT SUBSPECIES (Höglund et al., 2009; Cramp & Simmons, 1983): *Limosa limosa limosa*, the nominate race whose breeding range extends from Western Europe to Central Asia to the river Yenisey in Russia (Groen & Yurlov, 1999). Birds from Western and Central Europe winter in West Africa, while populations further east migrate to wintering sites in the Middle East and the east coast of India (Kuijper et al., 2006). In West Africa, the largest part of the birds winters in rice fields, while only a small numbers winter in natural wetlands (Piersma et al., 1996; Kuijper et al., 2006). Also on the Iberian Peninsula, rice fields are important staging areas for two months before the birds migrate towards their breeding grounds (Beintema et al., 1995; Kuijper et al., 2006; Gill et al., 2007).

Further east breeds *Limosa limosa melanuroides*, the Asian subspecies. They are considerably smaller, have a shorter bill and a more pronounced breeding plumage than the nominate species. They breed in scattered small populations in Mongolia, Northern China and in the East of Russia. These birds migrate to India, Indochina, Taiwan, the Philippines, Indonesia, Papua-New Guinea and Australia (Piersma et al., 1996; BirdLife International, 2006).

The Icelandic subspecies, *Limosa limosa islandica* is smaller than the nominate race, but still larger than the Asian subspecies (Piersma et al., 1996). Alike, it develops a more pronounced breeding plumage than the nominate race (Piersma et al., 1996, Gunnarsson et al., 2006a). As indicated by its name, this subspecies breeds almost exclusively on Iceland. Some pairs are reported on the Faroe Islands, the Shetland and Lofoten archipelagoes. Icelandic godwits winter in Great Britain, but increasingly also in Iberia and West Africa (Piersma et al., 1996).

Historical and current population trends and probable causes

Limosa limosa limosa

About 50% of the nominate population breed in The Netherlands. Black-tailed godwits used to breed in fen and bog areas (Beintema *et al.*, 1995) until the late 19th century, when many of these areas were lost or changed into agricultural grassland (Beintema *et al.*, 1995; Bijlsma, Hustings & Camphuysen, 2001; Gill *et al.*, 2007). Black-tailed godwits moved with the change and actually experienced high breeding success and drastic population increases in the beginning of this transition, most likely because of higher food availability for adult birds due to the increasing amounts of nitrogen applied to grasslands (Beintema *et al.*, 1995; Bijlsma *et al.*, 2001). Godwits used to be one of the main characteristic bird species in the Dutch landscape, with up to 240 000 individuals breeding in the country in the 1960ies (Piersma, 1986). But with ongoing advances in agricultural practice, extensively managed meadows made room for intensive agricultural fields (Beintema *et al.*, 1995). Since then the Black-tailed godwit population crashed dramatically to less than half its size (Thorup, 2006), and the decrease is still ongoing with an annual rate of –5% (Gill *et al.*, 2007).

The proximate cause for this decline is a reduced reproductive output, since adult survival remained high (Gill *et al.*, 2007; Roodbergen, Klok & Schekkerman, 2008; see also van Noordwijk & Thomson, 2008). Three most likely causes for the low reproductive output were identified (Gill *et al.*, 2007): (1) Lowering ground water levels, due to urbanization is presumably leading to lower food availability for adults and chicks (Kleijn & van Zuijlen, 2004; Verhulst, Kleijn & Berendse, 2007). (2) On intensively managed agricultural land, mowing dates advanced and godwits apparently breed now about two weeks earliest than fifty years ago (Beintema *et al.*, 1985; Schekkerman, 2008). Most agricultural activities but especially those that utilize large machinery like mowers, pose directly lethal danger to clutches and chicks (Beintema *et al.*, 1995). However, early mowing also has indirect effects on food availability for chicks, which depend on insects dwelling in the grass canopy: arthropod abundance drops steeply after mowing to very low levels, leading to high starvation risks for chicks that escaped the mowing blades (Schekkerman & Beintema, 2007). (3) Natural predation risk for clutches and chicks likely increased during the last decades (Bijlsma *et al.*, 2001; Teunissen *et al.*, 2005). This may partly be due to early mowing which leads to reduced coverage (Schekkerman, Teunissen & Oosterveld, 2009), but can also be due to a possible increase in birds of prey and small carnivorous mammals (Teunissen *et al.*, 2005; Teunissen *et al.*, 2008). The importance of each of these causes may differ locally, but the general trends on both, reserves and unprotected areas are similar and negative (Gill *et al.*, 2007).

Not much is known about the part of the nominate population that breeds in Central- and Eastern Europe and Russia (Groen & Yurlov, 1999). Although some countries report slightly increasing (albeit small) populations, the major part of the population, stemming from Russia and Belarus (appr. 80 000 individuals), is decreasing (Thorup, 2006).

Limosa limosa islandica

The Icelandic godwit population has undergone a very strong increase from 1900 until today, from 3000 to 75 000 individuals (Gunnarsson *et al.*, 2005a; Gill *et al.*, 2007). This increase happened concomitant with a range expansion from a restricted area in South-west Iceland to all over lowland Iceland (Gunnarsson *et al.*, 2005a). The reasons for this population increase are not fully understood. However, in the beginning of the last century, a period of rapid climate warming occurred simultaneously with a, most likely related, increase in available habitat in Iceland (Gill *et al.*, 2007). The climate warming is thought to have led to increased food availability, and godwits continuously occupied marshes and dwarf-birch bogs all over Iceland (Gill *et al.*, 2007). The estimates for adult survival of Icelandic godwits are similar to those of the nominate race, however, reproductive output estimates are considerably higher (Gill *et al.*, 2007). It was suggested that the drivers of the population increase in Iceland may be similar to the ones leading to the population rise early last century in The Netherlands, and this would suggest that further advancement of agricultural industry in godwit breeding habitat in Iceland might have negative effects (Gill *et al.*, 2007). The range expansion followed the pattern of a buffer effect: traditional sites, considered to be of high quality, were filled up first and over time, more and more new sites and also sites of lower quality, marsh and dwarf-birch bog habitat, got populated (Gunnarsson *et al.*, 2005a; -b).

Limosa limosa melanoroides

Not much is known about the Asian subspecies. Population trends are estimated accordingly to winter counts in Australia, where an estimated 50% (80 000, as of 1993) of the subspecies winters (Watkins, 1993). Also here, a rapid population decrease was observed (BirdLife International, 2006).

The above described population trends together have led to a population decline of approximately 30% of the global population within the last 15 years. In 2006, the IUCN red list of endangered species listed the black-tailed godwit as “near threatened” (BirdLife International, 2006).

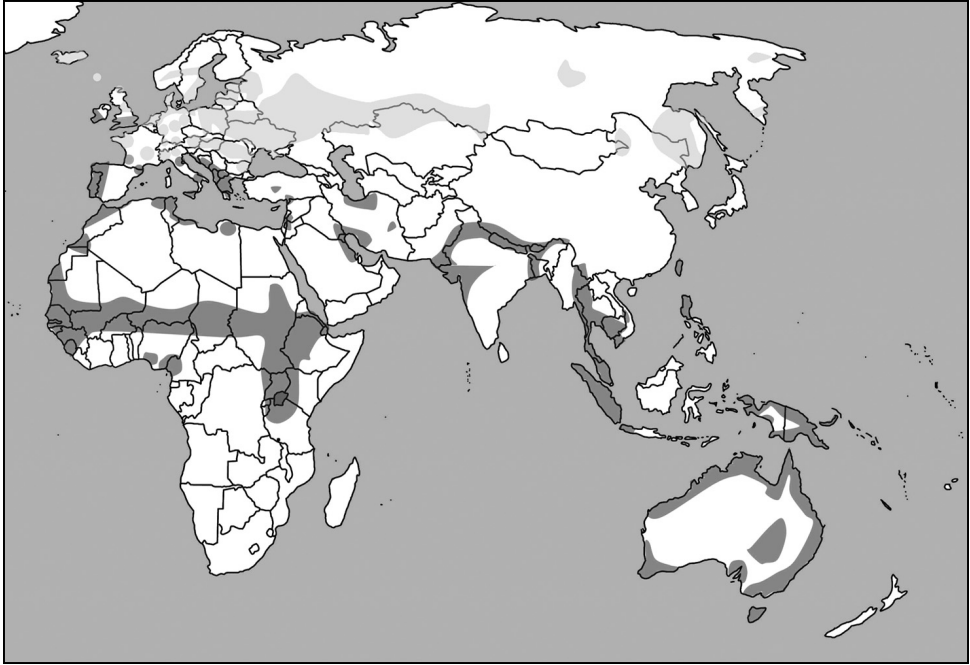


Figure A.1: Breeding (light grey) and wintering (dark grey) range of the Black-tailed godwit (*Limosa limosa*).

Two

Space use by black-tailed godwits *Limosa limosa limosa* during settlement at a previous or a new nest location

Valentijn van den Brink, Julia Schroeder, Christiaan Both,
Pedro M. Lourenço, Jos C.E.W. Hooijmeijer & Theunis Piersma

Capsule

Black-tailed godwits first return to the nest location of the previous year, even when moving to a different nest location later that season.

Aims: To examine the use of space by black-tailed godwits during the two months before egg-laying to two weeks afterwards.

Methods: We compare the spatial distribution of sightings of eventually site-faithful birds with birds that changed nest location, and relate this to the change of the distance to their previous year's and current nest-site in the period until egg-laying. Using a log-likelihood model we establish how the differences in distance to the respective nests change over the course of the season.

Results: All birds were observed first near their previous year's nest-site, and remained there for most of the pre-laying period. Birds that subsequently changed nest location made the move only about five days before egg-laying and were more wide-ranging earlier on.

Conclusion: The return to the previous nest-site suggests that a decision to move is made only after considerable time investment near the previous nest-site. This indicates that site-faithfulness in Black-tailed Godwits is conditional on experiences after return to the nesting area.

Introduction

CHOOSING A GOOD NEST LOCATION is crucial for successful breeding. Migratory birds, perhaps arguably, are more time- and energy-constrained than resident birds (Farmer & Wiens, 1999; Nolet & Klaassen, 2005; Drent, Fox & Stahl, 2006). For them, an early acquisition of a high quality breeding site as soon as possible after arrival on the breeding grounds may be especially important. Site-faithfulness would help birds to settle more rapidly, and several migrant shorebirds indeed show great site-faithfulness (Groen, 1993; Jackson, 1994; Flynn, Nol & Zharikov, 1999). Nevertheless, the decision to use the previous year's nest location again should be conditional on both the previous experiences (breeding success leading to return to the same place, Oring & Lank, 1982) and the conditions encountered after return (Dale *et al.*, 2006). For a passerine and a seabird there is evidence that breeding site selection is related to experiences in the previous season (Boulinier *et al.*, 1996; Pärt & Doligez, 2003). The behavior that precedes the decision of where to breed is called prospecting: it provides a means to gather information about the quality of possible breeding sites, and so-called 'public information' is one source of relevant knowledge (Danchin *et al.*, 2004). The quality of a breeding site can be judged by food availability, the absence or presence of nest predators and/or the mere presence or the breeding success of conspecifics, in the latter case certainly assessed in previous seasons (Danchin, Boulinier & Massot, 1998; Ward, 2005; Parejo, Oro & Danchin, 2006).

One component of prospecting would be movements near and far from potential nesting sites. Here we present a study of such movements in black-tailed godwits *Limosa limosa limosa* in a grassland area in the northern Netherlands. Mapping of individually color-marked birds over a two-month period enabled us to document the ranging of individuals that remained either faithful to the nest location of the year before, or actually changed nest-site. While most studies focus on events in previous years influencing the decision of whether to 'stay or go', we use individual movements before the establishment of a nest location and the start of egg-laying. We capitalized on a situation (perhaps due to early overgrazing by geese, see Discussion) where many godwits decided to change nest location from the one used in the previous year.

Study area and methods

THIS STUDY WAS CARRIED OUT in the northern part of the Workumerwaard, The Netherlands (52°59'N, 5°24'E), an area of 300 ha that includes extensively managed meadows in a polder, called Binnenwaard, and a grassy marshland area, called Buitenwaard, bordering Lake IJsselmeer (Fig. 2.1A). The polder is intersected by a road and the different meadows are separated by canals. The polder and the shoreline marshland are separated by a low dyke and a 7 m wide canal. The meadows in the polder are managed according to agricultural nature-management schemes, in which only dung and manure are used to the exclusion of artificial fertilizer, and earliest

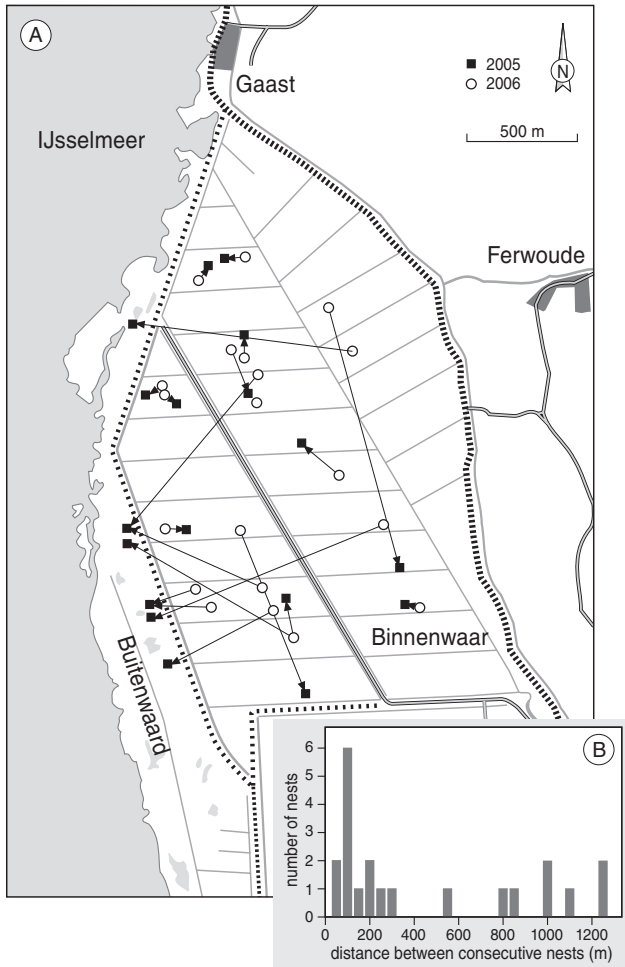


Figure 2.1: (A) Map of the research area, showing nests of individual black-tailed godwits for which nest-sites were known in 2005 and 2006. The lines connect nest-sites of individuals in both years. (B) Distances between nests of the same individuals for 2005 and 2006 in 50 m intervals.

mowing dates are 8 or 15 June. The shoreline marshland is managed as a nature reserve where the public are not allowed and mowing is limited to the dyke. The reserve is not fertilized, and cows and horses graze there at low density in summer. The study area harbours one of the highest densities of breeding black-tailed godwits in The Netherlands (Teunissen & Soldaat, 2005). In 2004 and 2005, 123 adult breeding black-tailed godwits were captured on the nest and received easily readable individual color-ring combinations consisting of four rings with four possible colors (white, yellow, red or blue) and a yellow flag (University of Groningen, 2008). On the basis of a small blood sample taken from the brachial vein, birds were sexed by molecular methods (Schroeder *et al.*, 2008a).

From March to May 2006, at least three times per week, both the Binnenwaard and Buitenwaard were searched for godwits and the locations of individually color-ringed birds were mapped to the nearest 50 m on a grid. Arrival dates were defined as the first day in 2006 that an individual was seen in the study area. In both 2005 and 2006, nests were searched by local volunteers of the meadow bird conservation group Fûgelwacht Warkum, who reported approximate locations to us. We revisited the nests and determined exact positions with handheld Garmin GPS 12 devices to the nearest 2 m. We used three different methods to link individual birds to their nests. Battery-powered digital video-cameras (Technaxx C-2000) were set up 1.5 m away from each nest and left there for 45 minutes. Most breeding birds returned to their nests soon after we left the meadow, and this allowed us to read the color rings of the bird on the video. In cases where no birds were recorded on the nest, we tried again, up to three times. In the rare cases where no birds would return to a nest with a video camera placed nearby, we tried reading the ring combinations from a hide. We further linked birds to their nest by recapturing adults on the nest. These efforts yielded a total of 21 individual birds for which the nest-site was known in both 2005 and 2006. These were mostly single birds within pairs. There were three pairs in 2006; one pair in 2005 were not together in 2006, and one pair we are certain had remained a pair between the two years, but whether the third pair was also together in 2005 is unknown. Leaving out either of the two mates in the pair that moved together, to avoid possible pseudoreplication, did not change the outcome of the statistical analyses qualitatively. To ensure the fullest possible presentation of movements, we left them in.

A total of 163 observations were available for these 21 birds. No observations of the period after hatching or loss of the nest were used. Birds were almost never observed after nest loss, which suggests that they left the area quickly. The number of observations per individual varied widely, from 1 to 22 (mean of 8.1 observations per bird \pm 1.2 se). For an indication of the accuracy of our estimates of arrival times, we calculated a resighting probability by averaging all individual probabilities to resight a bird after they were known to have arrived in the study area. The probability to resight an individual on any single day, when averaged over the whole study period for all birds, was 0.24 (\pm 0.02 se, $n = 20$).

For each observation, we determined the number of days prior to laying the first egg for that individual. For all analyses we then used the number of days from the moment of observation until the start of egg-laying; for example an observation made on day 12 would mean it was made 12 days before the nest of that individual godwit contained an egg for the first time. In our analysis of individual space use, relative to birds' former and future nests, we used only the distances of the individual observations before start of egg-laying to either the former or present nest locations in 2006. We emphasize that not all birds were observed for the same period of time prior to egg-laying, due to individual differences in timing of arrival and the timespan between arrival and the start of egg-laying.

Lay dates were estimated by measuring the degree of buoyancy of the eggs, which is related to the incubation stage (van Paassen, Veldman & Beintema, 1984; Liebezeit

et al., 2007). We used a correction factor for nests found when they were still incomplete to ensure that in the analysis ‘lay date’ is the start of egg-laying, and not the start of incubation. Assuming that it took five days to complete a clutch, and that there were four eggs per clutch (Beintema *et al.*, 1995), nests found with one, two or three eggs were assumed to be incomplete and the lay date was corrected to one, two or three days prior to finding the nest, respectively. All dates used are April days, where 1 April is day one. Based on Groen (1993), who showed that 80% of black-tailed godwits build their nest within 250 m of the nest location in the previous breeding season, *movers* denote birds that either moved more than 250 m away from their former nest-sites or that changed breeding location to the Buitenwaard. *Stayers* are birds that nested in 2006 within 250 m from the nest location in 2005 (Fig. 2.1B shows the frequency distribution of the distances between consecutive nests).

To establish whether the difference in distance to nests between movers and stayers was statistically significant, the statistical software program MLwiN (Version 2.02, 2005) was used to perform hierarchical linear models (Bryk & Raudenbush, 1992). These models take the nested relationship of repeated measures of individuals on different days into account. In our analysis we used two levels: individual and repeated measures. We used the standardized distance to the future nest-site as dependent variable. The standardization was calculated by simply subtracting the mean distance of all observations from the actual distance of each individual observation. This centered the values on a mean of zero. Predictor parameters were removed successively from the full model, starting with the least significant highest interactions. Significance was tested using the increase in deviance, which follows a chi-squared distribution. As predictor variables we used ‘days until initiation of the new nest’, ‘sex’ and ‘mover–stayer’, with stayer as reference. To account for intercorrelation of repeated observations of the same individual, ‘individual bird’ was added as a random factor into the model.

Results

IN 2006, THE AVERAGE ARRIVAL DATE of our focal birds was 25 March (± 1.5 days se, first arrival on 12 March); no differences were apparent between the sexes (Student’s *t*-test, $t = 0.4283$, $df = 19$, $P = 0.34$). Returning black-tailed godwits were first observed significantly closer to their previous nest location than to their future nest-site (paired *t*-test, $t = 2.66$, $df = 20$, $P = 0.01$), with 136 m (± 48 se) and 415 m (± 91 se), respectively, for the 2005 and 2006 nest locations. The average distance to the future nest-site for the whole period was 390 m. Again; there were no differences between the sexes ($t = 0.37$, $df = 19$, $P = 0.72$). Sex did not show an effect on the distance to the future nest-site in any of the models we tested. Of the 21 birds for which exact nest locations were known in the two years, nine were movers and 12 stayers (Fig. 2.1). There is no difference in number of observations per bird between the groups (*t*-test, $t = 1.19$, $df = 19$, $P = 0.25$).

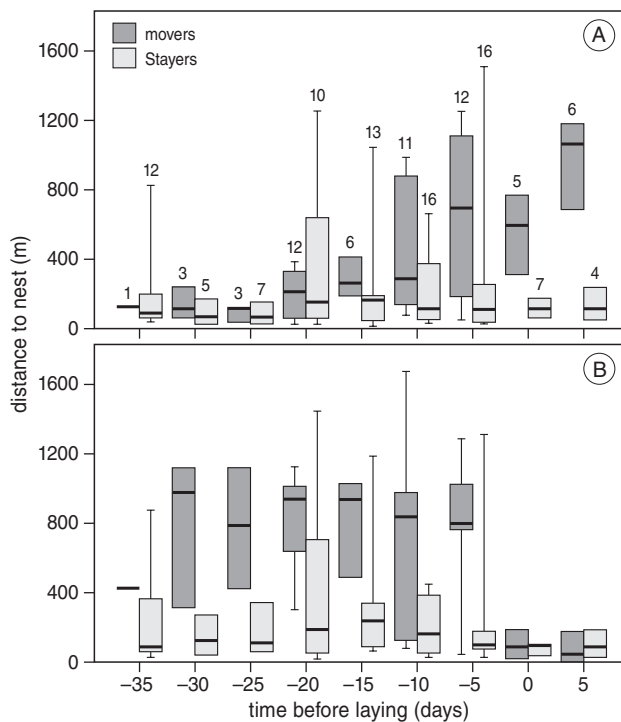


Figure 2.2: The effect of days until laying on the distance individual black-tailed godwits observed from their previous (2005) nest-site (A) and future (2006) nest-site (B) for birds that moved less than 250 m between years (light grey) and more than 250 m (dark grey). Indicated are median, 25–75% boxes and 95% confidence intervals. The observations are pooled in five-day periods. Above each box the number of observations in that period is shown. The graph is cut off at five days after the start of incubation, since movers and stayers both stay close to their nest during incubation.

Table 2.1: Distance to future nest-site in relation to days until laying, and whether individuals moved nest-sites.

| Parameter | Standardized distance to nest | | |
|--------------------------|-------------------------------|-------------------|-------|
| | β (SE) | χ^2 (df = 1) | P |
| Intercept | -0.50 (0.21) | 5.53 | 0.02 |
| Moved | 0.50 (0.29) | 2.99 | 0.08 |
| Days from laying | -0.003 (0.007) | 0.14 | 0.71 |
| Moved * Days from laying | -0.04 (0.01) | 11.02 | 0.001 |

The table gives the most parsimonious log-likelihood model ($-2 \log$ -likelihood value = 386.17) constructed in MLwiN and showing the effect of moving/staying on the standardized distance to the future nest. In this mixed model individual identity was included as random effect. ‘Moved’ is a categorical variable to distinguish between birds breeding within 250 m of the previous nest or further than 250 m. ‘Days from laying’ is the number of days until the start of egg-laying by an individual bird.

When grouping observations into five-day periods (Fig. 2.2) it is clear that all birds arrived close to their breeding site of the previous year. The movers seemed to start moving away from these sites 15 to 10 days prior to laying (Fig. 2.2A), but were found close to their new nest-site only around the time of egg-laying (Fig. 2.2B). In our final model (parameters displayed in Table 2.1), the interaction of 'days until laying' and 'movers and stayers' was found to be significant, while sex was dropped from the model. This model showed that before nest initiation, the movers were seen further away from their 2006 nest-site (mean 723 m \pm 407 se) than stayers (mean 283 m \pm 398 se; $t = -5.0$, $df = 161$, $P < 0.0001$), and this distance declined with date only for the movers (Fig. 2.2b). This indicates that the movers ranged over increasingly greater distances from the previous breeding site than the stayers before they settled.

During incubation, the two categories (movers and stayers) did not show significant differences ($t = 1.41$, $df = 10$, $P = 0.19$) in average distance from their actual 2006 nest location (142 m \pm 224 se). The average is strongly influenced by a single observation of a bird seen at 1000 m from its nest; without this observation the average distance was 98 m (\pm 19.5 se).

Discussion

UPON ARRIVAL on the Dutch breeding grounds, black-tailed godwits were first observed closer to the breeding site of the previous breeding season than to the site eventually used in the current year, suggesting that their first choice of breeding site was that of the previous year. Birds that were site-faithful remained there, but birds moving to a more distant nest-site did so only about five days before the start of egg-laying. This would be expected given the high nest-site fidelity in black-tailed godwits (Groen, 1993), but the relatively high frequency of movements in our study needs explanation.

An obvious explanation for the greater distance moved by the movers prior to breeding might be that we did not observe all nesting attempts, and that the nests we observed for the movers were replacement clutches. Although this is possible, the two groups did not differ in arrival date on the breeding grounds (t-test, $t = 0.90$ $df = 19$, $P = 0.38$), nor in the date of clutch initiation (t-test, $t = 0.09$, $df = 19$, $P = 0.93$). Therefore we have no reason to believe that we missed more first clutches in one of the two groups.

It is possible that the old breeding site is used as a meeting place for partners. Black-tailed godwits are socially monogamous, but pairs often winter far apart, as has been shown for the Icelandic black-tailed godwit *Limosa l. islandica* (Gunnarson *et al.*, 2004). After arrival on the breeding grounds, there is usually only a brief opportunity for migratory birds to reunite with the partner before the start of breeding (Choudhury, 1995). Therefore, a fixed location to reunite could prevent the need to spend precious time searching for the partner and this would allow for earlier breeding.

After reuniting, the godwits might prospect the suitability of the breeding area, using some sort of cue predicting its future condition. Food availability could be used as a possible cue since timing of breeding is often matched with timing of food availability for chicks (Hochachka, 1990; Thomas *et al.*, 2001; Dunn, 2004). For black-tailed godwits insect abundance during chick rearing is an important component of nest-site suitability (Schekkerman & Beintema, 2007). Another factor important for nest success of ground-breeding birds is nest predation, which is likely to be related to vegetation structure (Pärt, 2001; Whittingham & Evans, 2004). The Buitenwaard, where many movers went to breed in 2006, has a different vegetation composition from the abandoned nest-sites on the Binnenwaard. At least in 2006 the Buitenwaard had rougher and higher vegetation in the weeks before egg-laying. This contrasted with the situation on the Binnenwaard, especially in April 2006, which was grazed down to very short swards by Barnacle Geese *Branta leucopsis* (unpubl. data). We suggest that the birds departed from these short swards because nests would have been concealed from predators, and the phenology of insect availability may have been late relative to hatching. These movements to the Buitenwaard, however, did not result in higher reproductive success, because a large number of nests on the Buitenwaard were destroyed when the area was flooded during a storm.

An additional explanation for the observed long-distance breeding site changes might be mate change. It is often found that the divorce of bird pairs is not caused by a search for a better mate but rather by the search for a better territory. A number of studies report that it is the females that move, sometimes considerable distances, to a new territory (see references in Black, 1996). This change of territory, and of mate, is observed to have negative consequences for reproductive success in the first season after the move to the new territory. Unfortunately, our current sample sizes do not allow further analysis (of the nine movers, six were females, three were males; we observed only one certain divorce). In a year when the quality of the former breeding grounds has apparently deteriorated, we would expect that movers more often divorce to find a better nest-site and that females show the greater dispersal distances. The short time interval between movement and final egg-laying, however, suggests that movements were made by pairs together, rather than involving a switch in mates.

The higher variance in the distance to the nest before incubation shown by the movers (Fig. 2.2) indicated greater movement. This may be interpreted as prospecting in other areas. When they leave their previous breeding site relatively late to breed elsewhere, the movers might be making the best of a bad situation, as the most favoured territories will already be occupied by other birds. However, black-tailed godwits are semi-colonial breeders, who may cue on the presence of others to guide their nest choice and profit from each other by collectively defending nests against predators. This may allow them to move to other sites relatively late during the nesting season without too high a cost.

Acknowledgements

Petra de Goeij, Niko M. Groen, Rosemarie Kentie and all the students involved in the observations deserve credit here. We thank the conservation authority It Fryske Gea and the local farmers for their co-operation and for allowing access to their grounds. The assistance of the local volunteers of the Fûgelwacht Warkum for searching nests and reporting their locations to us has been invaluable. Dick Visser created the map of the study area. Mark Colwell and Simon Gillings provided valuable comments. This work was done under licence number DEC 4112B following the Dutch Animal Welfare Act Article 9. This work was financially supported by a set-up grant to T.P. from the University of Groningen, a grant to J.S. from the Schure-Beijerinck-Popping foundation and by BirdLife-Netherlands. P.L. was supported by a grant from the Portuguese Science and Technology Foundation.

Box B

Early breeding

Julia Schroeder, Jos Hooijmeijer, Christiaan Both & Theunis Piersma

Summary

Human impacts on the landscape have increased the penalties for black-tailed godwits laying their eggs too late, especially in the very intensive agricultural landscapes of The Netherlands. Thus, godwits have experienced a dramatic change of their fitness landscape, because the advance in mowing date made late clutches worthless destroying either eggs or chicks. To determine the driving forces of the recent population decline we study the individual variation in timing of breeding with respect to reproductive success in a population unaffected by mowing. Our results show that even in a low intensity agricultural area it is very important for godwits to breed early in the season.

“The importance of early breeding in black-tailed godwits (*Limosa limosa*)”;
Osnabrücker Naturwissenschaftliche Mitteilungen (2008) 35, 239–241.

Introduction

THE ENVIRONMENT OF MEADOWBIRDS in The Netherlands was subject to extreme changes during the last century (Beintema *et al.*, 1995). Detrimental conditions – in particular the advanced mowing date – are thought to have lead to the elimination of most offspring of birds that initiate breeding late in the season (for an overview see: Kruk, Noordervliet & ter Keurs, 1996). The temporal nature of this anthropogenic effect has obviously a different impact on late and early breeders. The negligible reproductive success of late breeders has been mentioned as a possible reason for the heavy decrease in many meadowbird populations (Kruk, Noordervliet & ter Keurs, 1997). To determine the driving forces of the population decline it can be revealing to look at the individual variation in timing of breeding with respect to reproductive success. Hence, in this project we ask: what determines why some godwits are early breeders and others not? And how does the timing of egg laying influence reproductive success in populations that do not suffer from advanced mowing dates?

The majority of the European population of black-tailed godwits breed in The Netherlands (Piersma, 1986; Beintema & Müskens, 1987); here they are still one of the more abundant breeding birds in what is left of the low intensity agricultural areas. Due to their relatively late breeding season black-tailed godwits are particularly vulnerable to early mowing dates (Kruk *et al.*, 1997). Despite much concern and debates, the decrease in numbers is going on unabated since the 1970's (Beintema *et al.*, 1995; Teunissen & Soldaat, 2005). We assume that the natural variation in individual timing of breeding correlates with individual quality and reproductive success, and thus can be related to the mechanisms causing the population decline. Therefore we test whether body condition and timing of egg laying are correlated with components of reproductive success in a population of the black-tailed godwit that is unaffected by mowing.

Methods

OUR STUDY AREA, the Workumerwaard in Friesland is a low intensity agricultural pasture complex of about 300 ha; mowing is delayed every year after 15th of June, thus not threatening godwit nests or chicks. In 2004, 77 nests were found. Eggs were measured, the average egg volume per nest was calculated (Romanoff & Romanoff, 1949) and lay day was estimated (van Paassen *et al.*, 1984). Early and late nests were defined by whether the birds initiated their nest before or after the mean lay day. Daily nest survival probabilities were calculated following Johnson (1979). 66 adult Godwits have been caught and individually color-ringed, weighed and measured. Sex of adults was determined biometrically (individuals with bill length > 99mm were classified as female), female condition was calculated as residuals from a linear regression of mass and tarsus-toe ($p < 0.001$). 107 chicks from 31 nests were caught, ringed and measured. We used the daily nest survival probability, average egg volume and average chick mass as estimates for reproductive success. Statistica® 7.0 was used for statistical analysis.

Results and Discussion

THE MEAN LAY DAY was the 24th of April. 40 nests were initiated before this day and 37 after. Daily nest survival probability was significantly different for early (0.996) and for late (0.989) nests (Fig. B.1A). Average egg volume per nest declined with lay day (Fig. B.1B). Average chick mass per nest was significantly lower in late chicks (Fig. B.2A). Females, which initiated nests early, were in a significantly better body condition than those that layed late (Fig. B.2B).

Daily nest survival probability was noticeably higher for early nests than for late ones. Further mean hatchling mass per nest was higher in earlier nests. Females in a good condition laid eggs early and low condition females initiated their nests later in

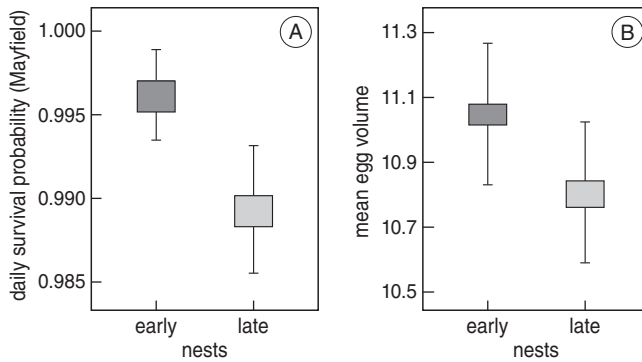


Figure B.1: A: Daily nest survival probability of early ($N = 40$) and late ($N = 37$) nests, z-test: $P < 0.001$, B: The seasonal decline of mean egg volume per nest ($N_{\text{early}} = 22$, $N_{\text{late}} = 22$, t-test: $P = 0.124$, $t = 1.568$). Bars represent 95% confidence intervals.

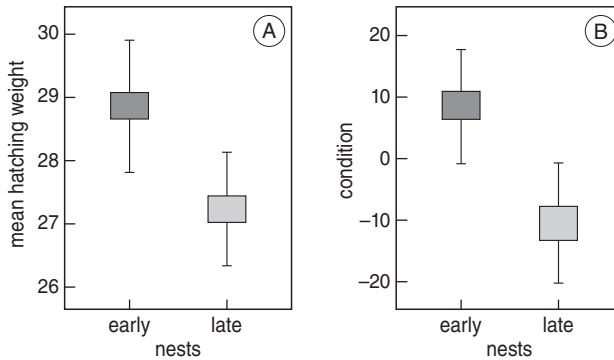


Figure B.2: A: The decline of mean hatchling mass per nest during the season ($N_{\text{early}} = 13$, $N_{\text{late}} = 18$, bars represent 95% confidence intervals, t-test. $t = 1.568$, $p = 0.019$); B: Condition of females with an early and late lay day ($N_{\text{early}} = 131$, $N_{\text{late}} = 11$, bars represent 95% confidence intervals, t-test, $t = 2.776$, $P = 0.011$).

the season. Thus, the individual timing of egg laying seems to be correlated with components of reproductive success. This decline over the season took place in a population unaffected by anthropogenetic influences such as mowing, thus can be considered as natural.

However, even late mowing dates can have devastating effects on chick growth, mediated by a lowered density of insects, which are the main prey items for growing godwits. The described pattern has been confirmed in 2005 (own data) and takes place in other populations of black-tailed godwits as well (Schroeder, Groen & Both, 2005). Seasonal declines in reproductive success are well known in many other bird species (Price, Kirkpatrick & Arnold, 1988), and black-tailed godwits seem to be no exception.

As not all birds are breeding early, we have to assume constraints on being early. Apparently, only females in a good condition can afford to initiate early nests. Individual body condition on the breeding grounds may also be affected by habitat quality – for instance mediated through food abundance – during non-breeding periods where they spend more than half of a years cycle (Marra, Hobson & Holmes, 1998; Norris *et al.*, 2003). In the Icelandic population of black-tailed godwits (*Limosa limosa islandica*) a link between the quality of wintering habitats and reproductive success was found. Birds feeding on high quality grounds in the winter were the same birds occupying the breeding grounds where high reproduction rates could be attained (Gill *et al.*, 2001).

This seasonal carry-over effect could be acting on the nominate race, too, with birds that winter on good quality sites being able to achieve a good condition in time to start breeding early. The conditions in wintering and staging areas of black-tailed godwits might affect foraging conditions and thus govern – via body condition – the feasibility to initiate early egg laying. As more and more droughts occur in the sub-Saharan wintering grounds and in southern Spain during the migration period, food abundance in increasingly dry habitats is expected to be rather low. In the context of a strong carry-over effect this could affect body condition on the breeding grounds and could be one of the reasons for the population decline.

Three

Caught between climate change and agricultural intensification: a grassland shorebird fails to adjust breeding date but does alter reproductive investment

Julia Schroeder, Niko Groen, Jos C.E.W. Hooijmeijer, Rosemarie Kentie,
Pedro M. Lourenço, Theunis Piersma, Hans Schekkerman & Christiaan Both

Abstract

Like other meadowbirds, black-tailed godwits (*Limosa l. limosa*) experienced drastic changes of their agricultural grassland breeding habitats in The Netherlands. Due to the advance of farming schedules associated with agricultural intensification and warmer springs, the seasonal food availability for chicks may have come to an increasingly early and rapid decline. Conditions affecting pre-laying food most likely improved. This was suggested to be the reason for an advancement of laying dates by two weeks between 1930 and 1976. We analyze hatching dates and hatchling mass of black-tailed godwits from The Netherlands during 1976–2007. Despite warming of springs, most likely leading to improvement of environmental conditions during laying, black-tailed godwits did not advance timing of breeding any further during this time. Instead, early females now lay larger, and late female smaller eggs than in the past. Heavier chicks were more likely to survive. After warm winters females laid larger eggs, probably because food availability for adults is high, and females probably are in good pre-laying condition. The reason that they have not continued to advance their laying date despite the ongoing strong selection for early breeding may be due to constraints in other parts of their annual cycle. The strong population decline of this bird may partly be due to this lack of advancement of breeding because this would have mitigated the severe reduction of reproductive success due to early mowing.

Introduction

IN SEASONAL HABITATS THE timing of reproduction is of major importance for reproductive success of birds (Lack 1950; Perrins 1970; Drent 2006). Because offspring survival depends on food resources, parents are expected to time their reproduction so that the time of maximum offspring food requirements coincides with maximum food availability. To achieve this, females may pay high costs, because they often should lay when food availability for themselves is low (Drent 2006). Hence the optimal laying date is a trade-off between these costs and benefits, and in most avian systems offspring number and quality declines with laying date, suggesting that many females breed after the peak in chick food abundance (Lack 1950; Perrins 1970; Drent & Daan 1980).

If fitness prospects of either the young and or the parents change seasonally, birds should optimize laying date, but also reproductive investment to environmental circumstances. In many species, clutch size declines over the season (Klomp 1970; Drent & Daan 1980; Daan *et al.*, 1990; Siikamäki 1998). If birds fail to adjust laying date to the phenology of the environment, they may reduce their clutch size even more (Both & Visser 2005); if they have an invariant clutch size, they can adjust the size of eggs if the value of late hatched chicks changes relative to that of early hatched chicks (Williams 2005).

Selection pressures for the timing of reproduction may fluctuate between years, e.g. due to stochastic weather, but can also change directionally in response to large-scale environmental changes. In many species, annual variation in average laying dates is related to pre-laying temperatures, with earlier laying in warmer springs (Dunn 2004). As a result, many species show directional trends in laying dates in response to recent climate warming (Crick *et al.*, 1997; Crick & Sparks 1999). These changes are expected in seasonal environments, because lower trophic levels tend to show earlier phenology patterns in warmer springs, and because the matching of food requirements with the peak in food availability requires earlier breeding (Visser & Both 2005; Both *et al.*, 2009). However, resource phenology for parents during egg production may change at a different pace than the phenology of offspring resources. This will change the trade-off between adult survival and reproductive output. Indeed, the responses of breeding dates to phenological changes in offspring food availability have been shown not to be sufficient in some systems, leading to an increased mismatch between timing of breeding and the food peak for chicks (Visser & Both 2005), followed by population declines (Both *et al.*, 2006).

Most of the responses in avian breeding phenology to a changing environment have been shown to be phenotypically plastic (Przybylo *et al.*, 2000; Charmantier *et al.*, 2008; Gienapp *et al.*, 2008). Nevertheless, species with longer delays between the onset of laying and the time of maximum food requirements for chicks are expected to have smaller capacity to respond because of limited predictability at the time of laying (van Noordwijk & Müller 1994; Both *et al.*, 2009). Further, life history theory predicts a trade-off between current and future reproduction (Stearns 1992; Bennett & Owens

2002; Brinkhof *et al.*, 2002), and in species that reproduce more than once during their lifetime, females may decide to invest little or even skip a year's reproduction if food availability is low during egg production (Erikstad *et al.*, 1998). Such a bet-hedging strategy will be maladaptive if the environmental variability that leads to smaller investments is not stochastic but unidirectional, and the better future that is bet on will never come (Pearce-Higgins *et al.*, 2009).

Birds not only have to cope with climate change, but at the same time they experience other directional changes in their habitat, often directly caused by humans altering habitat. Probably most challenging are the advances in the timing and intensification of agricultural activities for ground-breeding grassland birds in the north-temperate climate zone (Beintema *et al.*, 1995; Schekkerman & Beintema 2007; Schekkerman *et al.*, 2008). In meadow habitats the sequence of fertilizer application and mowing has advanced, increased drainage and fertilization levels and spring warming allowing farmers to have more and earlier harvests per season. Mechanical mowing exerts a strong selection pressure on ground breeding birds, because it destroys nearly all nests, kills chicks and sometimes even incubating parents (Schekkerman *et al.*, 2009). Chicks that escape the mowing machines experience a severe drop in insect food availability and cover, reducing survival prospects considerably (Schekkerman & Beintema 2007; Schekkerman *et al.*, 2009). Drier summers due to climate change may have the same effect on insect availability – the food for chicks (Pearce-Higgins *et al.*, 2009), while an acceleration of vegetation growth may advance the seasonal insect peak and reduce foraging success for late born chicks due to problems with vegetation density (Kleijn *et al.*, in press).

The same increased use of grassland fertilizer and warmer soils early in the season positively affect the abundance of belowground prey (Edwards & Loft 1982; Jordan *et al.*, 2004; Timmerman *et al.*, 2006), favoring adult birds when they arrive on the breeding grounds after spring migration. Food availability often relates to female nutritional state, which in turn positively influences laying date and reproductive investment (Bolton *et al.*, 1992; Ratcliffe *et al.*, 1998), enabling females to initiate larger clutches earlier (Nager 2006).

Indeed, there is support for a connection of laying date advancement in Dutch grassland-breeding shorebirds (black-tailed godwits *Limosa l. limosa*, northern lapwing *Vanellus vanellus*, redshank *Tringa totanus*, snipe *Gallinago gallinago*, ruff *Philomachus pugnax* and oystercatcher *Haematopus ostralegus*) with increased use of fertilizer during 1940-1976 (Beintema *et al.*, 1985), and in northern lapwing also with warmer springs and wetter winters during 1901-2003 (Both *et al.*, 2005). All three factors affect food availability during egg laying and may have enabled females to lay earlier. However, for all these species but northern lapwings, these conclusions are based on data from 1911-1973 (Haverschmidt 1963; Beintema *et al.*, 1985), from before the greatest change in climate.

Since then, agricultural practices have continued to intensify, nitrogen loading of the Dutch grasslands increased until the 1980s, mowing dates advanced and local spring temperature increased (Both *et al.*, 2005; Kleijn *et al.*, in press). In response,

grassland-breeding shorebirds either should continue advancing their timing of breeding, or should change the seasonal patterns of reproductive investment. That the Dutch black-tailed godwit population is in steep decline since the 1970s (Thorup 2006; Gill *et al.*, 2007; Schekkerman *et al.*, 2008) implies that these birds have not successfully adapted to the environmental change they experienced. Here, we explore how black-tailed godwits responded with breeding time and reproductive investment to climatic changes and associated changes in agricultural practice. For this we analyze data on laying dates, egg volume and hatchling mass from 1976-2007 for populations in The Netherlands, the major breeding area of the West-European godwit population.

Methods

Study species

From late February to early April black-tailed godwits return to their Dutch breeding grounds from staging areas in Iberia (van den Brink *et al.*, 2008). Upon return, adult godwits forage mainly on earthworms (Beintema *et al.*, 1995). Female godwits initiate nests from early April onwards (Beintema *et al.*, 1995). Replacement clutches after nest failure occur but are rare after mid-May (Schekkerman & Müskens 2000). The precocial chicks hatch in May or June and their diet consists of insects from the grass canopy, especially Diptera, many of which exhibit a seasonal abundance peak related to temperature, and are sensitive to dry spells in previous years (Beintema *et al.*, 1991; Pearce-Higgins & Yalden 2004; Schekkerman & Beintema 2007; Pearce-Higgins *et al.*, 2009).

Black-tailed godwits are strongly influenced by human activities, even in areas managed especially for them. From February onwards, depending on the weather, farmers apply fertilizers to the grasslands. At present, mowing starts in mid- to late April, during the time when black-tailed godwits incubate (Schekkerman *et al.*, 2008; Kleijn *et al.*, in press).

Data sets

We used four different datasets collected in The Netherlands over 30 years (Table 3.1). In all areas hatchlings were ringed in the nest (which is only possible on the day of hatching), and some of these chicks were recaptured later before fledging. The datasets are: (1) During 1976-1985, in a nation-wide Dutch chick-ringing program, data of 1480 hatchlings and 49 recaptured chicks were collected mainly by amateurs (Beintema 1995). (2) From 1984 to 1989, data on 966 hatchlings, 12 recaptured chicks and egg dimensions from 265 nests were obtained by NMG and coworkers in a nature reserve near Zaanstad (52°31'N, 4°47'E) (Groen & Hemerik 2002). (3) Data on 99 hatchlings were collected near Baarn (52°12'N, 5°19'E) from 1993-1995 (Schekkerman & Beintema 2007). (4) From 2004-2007, data on 764 hatchlings, 43 recaptured chicks and corresponding 207 nests with egg dimensions were collected in southwest Friesland (52°59'N, 5°24'E) (Schroeder *et al.*, 2008; van den Brink *et al.* 2008).

The oldest dataset was gathered unsystematically on several sites, while the other datasets come mainly from nature reserves or areas with agri-environment schemes specifically managed for meadowbirds, hence breeding areas of high quality. To reduce clutch mortality, the management regimes in these nature reserves mimic agricultural practices in the 1960s and 1970s with respect to grazing density and timing of mowing. Whereas conditions in the normal agricultural areas have intensified over the last 30 years, the conditions in the nature reserves have only done so moderately, and are more comparable to circumstances in the past. Furthermore, areas with high

Table 3.1: Annual sample sizes of eggs, nests with data on egg volume and hatchlings, total amount of hatchlings caught and recaptures at an age more than four days of Black-tailed Godwits breeding in The Netherlands. In the two last columns, annual parameter estimates of a linear mixed model of hatchling mass on date of season are given.

| data set | year | Nests with chicks and corresponding egg volume | hatchlings | recaptures | intercept | slope |
|----------|-----------|--|------------|------------|-----------|-------|
| BE | 1976 | NA | 91 | 4 | 28.91 | -0.02 |
| BE | 1977 | NA | 119 | 6 | 28.80 | 0.02 |
| BE | 1978 | NA | 258 | 15 | 28.47 | 0.04 |
| BE | 1979 | NA | 201 | 3 | 29.69 | 0.06 |
| BE | 1980 | NA | 98 | NA | 27.39 | 0.02 |
| BE | 1981 | NA | 132 | 9 | 27.45 | 0 |
| BE | 1982 | NA | NA | NA | NA | NA |
| BE | 1983 | NA | 54 | 2 | 28.31 | 0.02 |
| BE & NG | 1984 | NA | 385 | 8 | 28.38 | 0.02 |
| BE & NG | 1985 | 68 | 461 | 2 | 28.96 | 0.02 |
| NG | 1986 | 65 | 222 | 8 | 28.50 | -0.03 |
| NG | 1987 | 47 | 160 | NA | 28.58 | -0.01 |
| NG | 1988 | 44 | 145 | 2 | 28.72 | -0.03 |
| NG | 1989 | 41 | 120 | 2 | 27.66 | -0.04 |
| | 1990-1992 | NA | NA | NA | NA | NA |
| HS | 1993 | NA | 49 | NA | 28.07 | 0.01 |
| HS | 1994 | NA | 28 | NA | 27.89 | -0.02 |
| HS | 1995 | NA | 22 | NA | 28.54 | -0.02 |
| | 1996-2003 | NA | NA | NA | NA | NA |
| JS | 2004 | 32 | 86 | 8 | 27.84 | -0.06 |
| JS | 2005 | 54 | 160 | 8 | 28.33 | -0.04 |
| JS | 2006 | 39 | 154 | 2 | 27.83 | 0.03 |
| JS | 2007 | 82 | 364 | 25 | 28.33 | -0.04 |

godwit densities, and hence high quality, are likely to have provided most of the chicks caught by volunteers from the first study, as avoidance of these best sites seems unlikely. Therefore, we think that any difference in area quality can be neglected for the purpose of this study.

Data preparation and definitions

Egg volume, available for the years 1984-1989 and 2004-2007, was calculated as $\text{length} \times \text{width}^2 \times 0.52$ (Romanoff & Romanoff 1949). Godwits nearly always lay a clutch of four eggs (Schroeder *et al.*, 2009). Consequently, differential investment to clutches can mainly be achieved by female godwits varying egg volume and timing of laying. In birds, egg production is costly (Williams 2005) and can affect survival and/or future fecundity of females (Monaghan *et al.*, 1998; Nager *et al.*, 2001; Visser & Lessells 2001). Egg volume has been shown to reflect female nutritional status at laying (Amat *et al.*, 2001; Sanchez-Lafuente 2004; Karell *et al.*, 2008) and female godwits relatively heavy for their size lay larger eggs (Schroeder *et al.*, 2009). We therefore assume that average egg volume per nest is a useful index of female reproductive investment. Here we want to test whether females adjust reproductive investment to environmental conditions, but instead of egg volume we use hatchling mass, as we have a much larger dataset for this parameter which is closely related to egg volume (Hegyí & Sasvári 1998).

The rationale of the analysis is that hatchling mass, an indicator for egg volume and therewith female reproductive investment, is indeed a fitness correlate since it is indicative of chick survival, which is lowest during the first days after hatching and increases substantially later, with heavier chicks being more likely to survive (Schekkerman *et al.*, 2009). Hence the more a female invests in her eggs, the heavier her offspring and the higher her inclusive fitness.

Reproductive success is related to laying date in black-tailed godwits (Roodbergen & Klok 2008), and hence we analyze within and between year variation in laying date. Hatching occurs c. 23 days after the start of incubation (Cramp & Simmons 1983; Beintema *et al.*, 1995). Laying date was defined as the start of incubation, calculated by subtracting incubation duration from the hatching date or, if hatching date was unknown, by estimating the incubation stage of the eggs (van Paassen *et al.*, 1984; Liebezeit *et al.*, 2007).

Environmental covariates

We analyzed whether laying date and hatchling mass are affected by different environmental variables with ecological relevance. As most important factors we consider (1) the phenology of food abundance before and during egg-laying, (2) the date of first mowing because this affects food availability and causes direct mortality among nests and chicks, (3) weather during the chick period, affecting insect abundance and activity. Because we have no direct measures, we use approximations of annual variation in these variables based on temperatures collected at De Bilt (52.12°N 5.18°E), in the central Netherlands from 1901 until 2007 (www.knmi.nl). .

The climate variables we use as approximation for the factors named above are: (1) the date farmers are supposed to apply fertilizer, (2) the date that most meadows get mown and (3) temperatures during May that determine timing of insect availability for chicks (Pearce-Higgins & Yalden 2004; Nooker *et al.*, 2005). (1) Timing of fertilizer application depends on plant phenology, which is partly determined by the date a certain temperature-sum during late winter/early spring is reached. These annual temperature-sums are the sum of all average daily temperatures (= mean of daily minimum and maximum) for days with positive temperatures from the first of January until the focal date. Farmers are advised to use the date when a temperature sum of 180°C is reached to determine the optimal timing of the first fertilizer application (Kruk *et al.*, 1996). It is a description of plant growth and likely affects invertebrate abundance and phenology (Jordan *et al.*, 2004; Timmerman *et al.*, 2006).

(2) Farmers determine the optimal date of mowing based on features of meadows, and since individual meadows differ, the first mowing varies over a period of several weeks. A general predictor of the timing of mowing is the date when the temperature-sum reaches 890°C, when 50% of the grassland area is mown (Kleijn *et al.*, in press). We use this variable to indicate when food availability for chicks will strongly decline due to mowing.

We also tested other weather variables (late winter/early spring air temperatures and precipitation, similar to Both *et al.*, 2005). None of those revealed interesting patterns and therefore we only present analysis on the above-mentioned variables.

Statistical analyses

We used mixed-effect models with a variable representing year (cohort) as random factor accounting for variation in annual sample sizes.

To show that hatchling mass conveys the same information as egg volume, a linear mixed model (LMM) was employed with average chick mass per nest, dataset and year as main effects and cohort as random effect on the intercept. Data on egg volume and corresponding chick mass was available for 472 nests in 1985-1989 and 2004-2007 (Table 3.1). Both variables were averaged per nest.

To determine what influences hatching date, we used a linear model with median annual hatching date as dependent variable and the climate variables and year as explanatory variables. To examine the relationship between hatchling mass and date, and its change over time, we applied a LMM with hatching date of individual chicks, year (as a continuous variable) and the interaction of both covariates, and cohort as random effect on the intercept. For most datapoints, we have no information on nest affiliation. To further examine what influences the change of the seasonal decline, we used step-wise deletion of non-significant terms from a linear model with the parameter estimate of the slope of the former model as dependent variable, and all climate variables and year as covariates. Because we do find a change in the covariation between hatchling mass and hatching date over the years, we examine whether this is due to early chicks increasing and/or late chicks decreasing in mass over time. We do this by examining trends for the earliest 10% and the latest 10% chicks. The former

also circumvents the problem of replacement clutches, which likely to contain smaller eggs. We tested with linear models for association between climate variables and the average hatchling mass of the 10% earliest and latest born hatchlings of each year.

We used R.2.7.1 statistical software (R Development Core Team 2008). The lme function (nlme package) of was employed for fitting LMM and the lm function (base package) was used for fitting linear models. We selected the most parsimonious model by AIC (Burnham & Anderson 2002). For LMM, we report parameter estimates \pm standard errors and the significance level of each coefficient when all other mentioned fixed effects are in the model. For linear models, we used step-wise deletion of non-significant effects until only significant ($p < 0.5$) effects remained.

Results

Egg volume

Egg volume and hatchling mass did not differ between the two datasets where egg volume was available (t -test: $t_{EV} = -0.63$, $P_{EV} = 0.53$; $t_{HM} = -0.89$, $P_{HM} = 0.38$, $N = 472$). Larger eggs hatched larger chicks (Fig. 3.1). The best model explained hatching mass with egg volume only ($\beta_{EV} = 0.53 \pm 0.02$, $t = 23.77$, $P < 0.001$, $N = 472$, $R^2 = 0.54$). Neither year nor dataset remained in this model (both fixed effects $P > 0.25$ when in the model).

Changes in temperature

For all three climatic variables there is an initial phase of no directional change up to ca 1975, after which all show a trend towards warming (see LOWESS trends lines in Figs 3.2A-C). We concentrate here on the trends from 1976 onwards because our godwit data starts here, but it should be highlighted that before this year godwits have shown a steady advance in their breeding dates, without an apparent increase in the temperatures they experienced before laying (Beintema *et al.*, 1985).

Since 1976, average May temperatures increased by $0.06 \pm 0.03^\circ\text{C}$ SE per year ($F_{1,30} = 4.58$, $P = 0.04$). There was a trend for the temperature-sum 180°C to be reached earlier every year ($\beta_{t\text{-sum } 180} = 0.81 \pm 0.41\text{SE}$, $F_{1,30} = 3.92$, $P = 0.06$) and the temperature-sum 890°C advanced significantly ($\beta_{t\text{-sum } 890} = 0.69 \pm 0.20\text{SE}$, $F_{1,30} = 11.66$, $P < 0.001$). The quadratic tem was rejected in all three models ($P > 0.90$). Before 1976, the temperature-sum date 180°C and average May temperatures were not correlated with each other ($\beta_{\text{may}} = 0.009 \pm 0.007\text{SE}$, $F_{1,73} = 1.88$, $P = 0.17$), implying that warmer winters were not more often followed by high May temperatures and therefore could not be used as a cue for the prediction of spring weather. In contrast, post 1976, the temperature-sum date 180°C was positively correlated with average May temperatures ($\beta_{t\text{-sum } 180} = 0.06 \pm 0.03\text{SE}$, $F_{1,30} = 6.09$, $P = 0.02$).

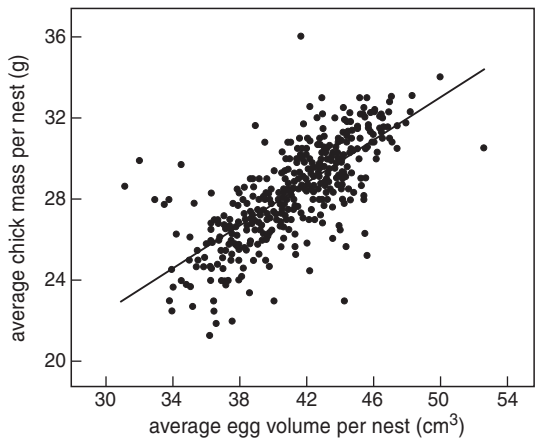


Figure 3.1: The relationship between average egg volume per nest and average chick mass in black-tailed godwits breeding in The Netherlands.

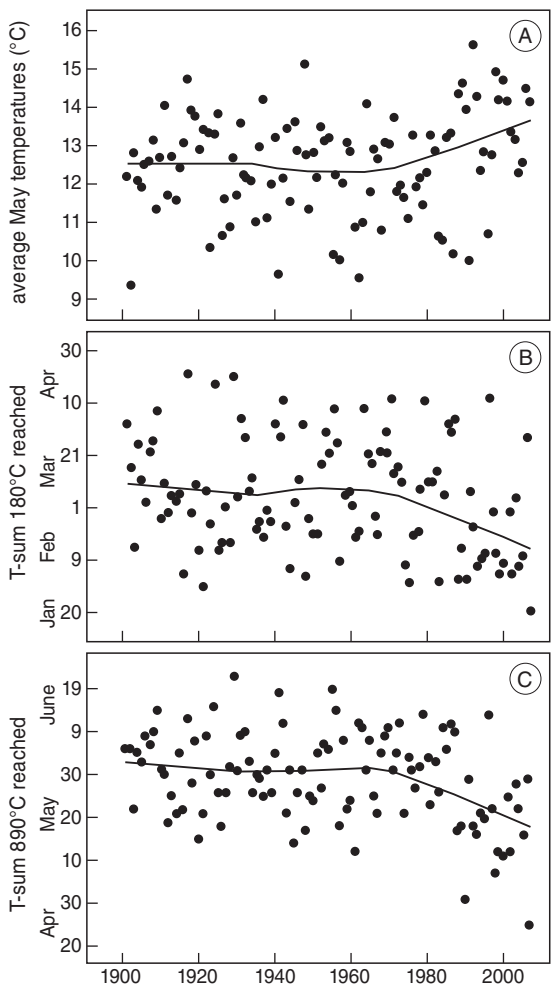


Figure 3.2: The average May temperature (A), the date the temperature-sum of 180°C (B) and 890°C (C) were reached over the course of time (1901-2008). Lines represent locally weighted regression scatter-plot smoothing (LOWESS, $f=1$) to enhance visual perception.

Trends in hatching date

The increasing temperatures during late winter and spring did not lead to a significant advancement in annual median hatching date from 1976 to 2007 (Fig. 3.3A, $F_{1,18} = 0.06$, $P = 0.81$). To exclude the possibility that in more recent years more second clutches obscured a possible advance of laying date, we tested only the earliest 10% of all nests each year. To exclude the possibility that the national decrease in population size may have concealed a possible advance (Tryjanowski & Sparks 2001), we also tested for the first 20 and 30% of all annual nests and of those between 20 and 80%. We did not find evidence for an advancement of the average hatching date for any of these subsets (all $P > 0.77$). Surprisingly, median hatching dates of godwits were earlier when May temperatures were low and later when May was warm (Fig. 3.3B). Only May temperature remained in a model explaining median hatching date ($\beta_{\text{may}} = 1.75 \pm 0.74\text{SE}$, $F_{1,18} = 5.56$, $P = 0.03$) and temperature-sum 180°C and 890°C and year were removed from the model (all $P > 0.49$).

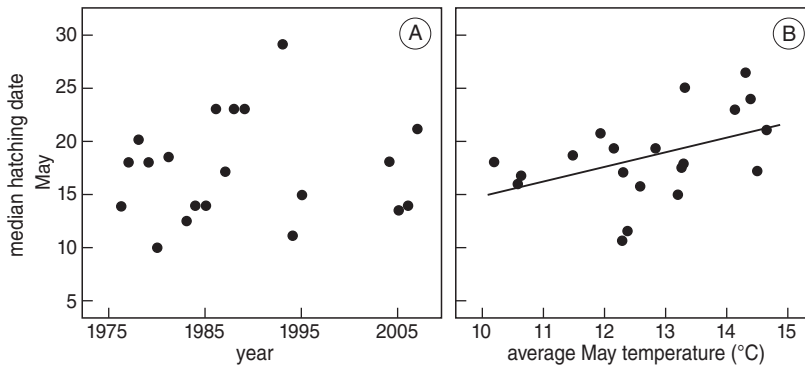


Figure 3.3: The average annual hatching date of Dutch black-tailed godwits (A) over the course of time and (B) in relation to average May temperatures. For statistics see text.

Temporal changes in hatchling mass in relation to climate

The seasonal decline in hatchling mass became stronger over the years (Fig. 3.4A, B). The best linear model explaining hatchling mass included the interaction between year and hatching date (Fig. 3.4A, LMM: $\beta_{\text{hatching date}} = -0.25 \pm 0.07\text{SE}$, $t = 5.13$, $P < 0.001$; $\beta_{\text{year}} = 0.05 \pm 0.03\text{SE}$, $t = 3.79$, $P = 0.20$; $\beta_{\text{hatching date} \times \text{year}} = 0.01 \pm 0.0001\text{SE}$, $t = -5.13$, $P < 0.001$, $N = 3309$). During 1976–2007 the effect of hatching date on hatchling mass was first positive, but in due course changed to negative: in recent years early hatchlings were heavier than late ones (Fig. 3.4B; $F_{1,18} = 6.27$, $P = 0.02$). We examined covariation of this change in seasonal decline with a linear model explaining the annual slopes of the previous linear mixed model with the climate variables and year as covariates. In addition to a linear year effect, we found an effect of the T-sum 180°C : after warmer winters hatchling mass declined more steeply with hatching date than after colder winters ($t\text{-}180^\circ\text{C}$: $F_{2,17} = 8.43$, $P = 0.01$, $\beta_{t\text{-}180} = 0.001 \pm 0.0001\text{SE}$, year: $F_{2,17} = 8.85$, $P = 0.01$, $\beta_{\text{year}} = -0.001 \pm 0.001\text{SE}$).

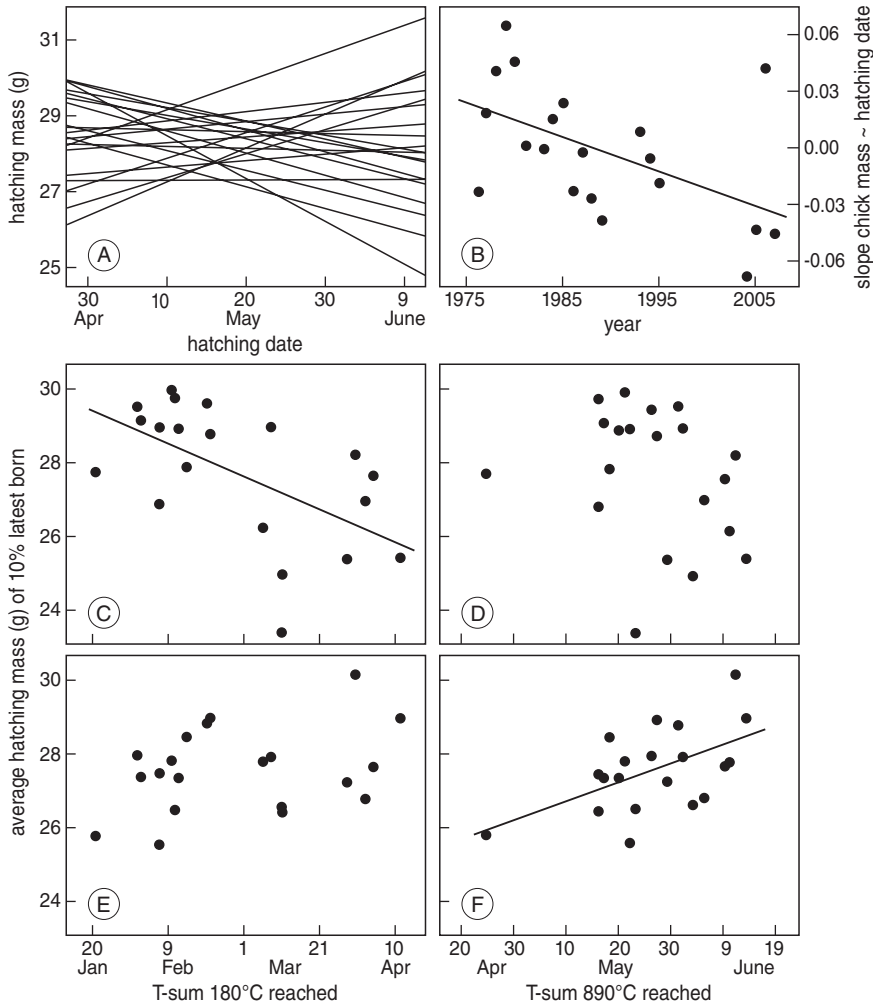


Figure 3.4: A: Annual regression lines of hatchling mass of black-tailed godwits in The Netherlands in relation to hatching date. B: The parameter estimates of those annual regressions are plotted against year. C, D, E, F: The average hatchling mass of the first (C, D) 10% and the last 10% (E, F) of all chicks born per year in relation to the date the t-sum of 180°C was reached (C, E) and in relation to the date the t-sum of 890°C was reached (D, F). For statistics see text.

A change in the seasonal reproductive investment pattern can arise because (1) hatchlings became both heavier early in the season and lighter later, (2) only became heavier early in the season, or (3) only lighter late in the season. The latter two explanations are expected to result in a change of average hatchling mass over the years. This did not happen ($F_{1,18} = 1.74$, $P = 0.20$). Under the latter two hypotheses we also expected the annual variance in hatchling mass to increase over the years, which we did not find either ($F_{1,18} = 0.19$, $P = 0.67$). Thus, over the years, early born chicks seem to have become heavier, and later born chicks lighter.

To get a better understanding of why birds increasingly lay larger eggs early and smaller eggs later in the season, we explore patterns between environmental correlates and the annual mean mass of either early or late hatchlings. We employed two linear models to correlate, respectively, (1) the annual average hatchling mass of the 10% earliest ($N = 307$) and (2) the 10% latest born chicks per year ($N = 150$) with climate variables and year. In the most parsimonious model with the earliest chicks (1), only the temperature-sum 180°C remained in the model, indicating that early hatchlings were heavier in years with warm winters (Fig. 3.4C, $F_{1,18} = 7.55$, $P = 0.01$, $\beta_{t\text{-sum}180} = -0.04 \pm 0.02$ SE). All other variables were removed from the final model (Fig. 3.4d, temperature-sum 890°C: $P = 0.10$, all other $P > 0.46$).

In the best model with the latest 10% chicks (2), only the temperature-sum 890°C remained, indicating that these late hatchlings are lighter in years with warm springs and earlier mowing (Fig. 3.4F, $F_{1,18} = 8.08$, $P = 0.01$; $\beta_{t\text{-sum}890} = 0.05 \pm 0.02$ SE). All other variables were removed from the best model (Fig. 4E, temperature-sum 180°C: $P = 0.10$, all other $P > 0.31$). Thus, the change in hatchling mass early in the season (1) is best statistically explained by the temperature-sum 180°C (adult food availability). The change in hatchling mass late in the season (2) is best explained by the temperature-sum 890°C (mowing dates). May temperatures (which may indicate food phenology for the chicks) did not explain the change in hatchling mass over the season.

Discussion

DURING THE LAST 30 YEARS black-tailed godwits experienced strong directional changes in their breeding habitat. Increasingly warm winters and early springs and associated advancing agricultural schedules and fertilizer inputs most likely led to earlier, and possibly higher food availability for adults (Edwards & Lofty 1982; Beintema *et al.*, 1985; Beintema *et al.*, 1995; Jordan *et al.*, 2004; Timmerman *et al.*, 2006). Surprisingly, this has not lead to earlier laying, contradictory to advancements of laying dates found in other waders (Both *et al.*, 2005; Pearce-Higgins & Yalden 2004), and the general effect of spring temperature on laying date among birds (Dunn 2004). But godwits did change other life-history traits: over the years they increased the hatchling mass of early born chicks (through laying larger eggs early in the season) but produced lighter chicks later in the season.

We do not know the strength of any selection on breeding date in the past, but in birds early breeders generally have higher fitness (Drent 2006; Verhulst & Nilson 2008) because of costs of early laying and/or constraints in food availability (Perrins 1970). One of the prime changes in selection pressures on timing of breeding in godwits is the advance of mowing dates. Mowing causes high mortality of eggs and chicks, and chicks that do survive experience a strong drop in food availability and shelter (Schekkerman & Beintema 2007; Schekkerman *et al.*, 2009). This change is very recent. Godwits advanced egg laying dates prior to 1975 most likely because food

availability before laying improved, but they failed to advance laying date in recent years, while selection for early laying intensified because of increasing chick mortality due to advancing mowing dates. The change in timing of breeding in earlier years, in response to food availability pre-laying, was most likely phenotypically plastic; many bird species react with earlier breeding to food supplementation (Drent 2006). It therefore indicates that in godwits, breeding time has a relatively large environmental component, which means that heritability is likely to be low. The latter is supported by relatively low repeatability of female godwit laying date (<0.08 , $N = 38$, $N_0 = 2.3$; unpublished data). This together with the realization that godwit generation times are rather long (5–14 years, Roodbergen *et al.*, 2008) let us believe that an evolutionary response to selection by mowing after such a short time is unlikely. While a response with timing of breeding to the food situation for females is possible (and likely has happened in the past), a response to the strong selection for early breeder by mowing is (now) possible only on a phenotypic basis. However, for this to take place, females would need a reliable cue to predict conditions (food abundance for chicks and mowing dates) at the time of egg production. The weather during late winter did not predict spring conditions pre-1976. Therefore, to use this environmental cue nowadays (as winter condition do predict spring climate now), godwits would have needed to learn to do so during the past 50 years, which apparently did not happen.

However, pre-1976, godwits did show a response to improved conditions for adults during egg production. The previous advancement of laying date in black-tailed godwits during 1940 – 1975 was observed on the basis of ringing dates of chicks (Beintema *et al.*, 1985); and data retrieved from Haverschmidt (1963) show that the date on which the first godwit egg was found in Friesland (the Dutch province that harbors most breeding godwits) advanced by two weeks from 1930 until 1960 (Fig. 3.5). This advancement was explained by the increased use of fertilizer on grasslands and the resulting improved food situation for adults, possibly enabling females to lay earlier, but not by an change in climate, meaning females likely responded to the food situation for themselves rather than to the expected one for their chicks (Beintema *et al.*, 1985). We found that laying dates of black-tailed godwits did not advance any further after 1976 (Fig. 3.3A), while simultaneously temperatures in The Netherlands began to rise and agricultural schedules advanced further. Kleijn *et al.* (in press) also conclude, on the basis of a different dataset, that laying dates did not advance since 1980, suggesting that the non-advancement of timing of breeding is a robust result. Assuming that ongoing changes in agricultural schedules further led to earlier and higher food availability for laying females, why did godwits not advance laying dates further?

Black-tailed godwits also advanced their arrival date by three weeks from 1930 until 1962 (Fig. 3.5, Haverschmidt 1963). Since then, arrival times have not changed much. In recent years, the first godwits are still seen in early March (van den Brink *et al.*, 2008; Kleijn *et al.*, in press). It is possible that the advance in breeding date is only possible if accompanied by an advance in arrival date, and that circumstances during other times of their annual cycle prevent earlier migration or are limited in flexibility,

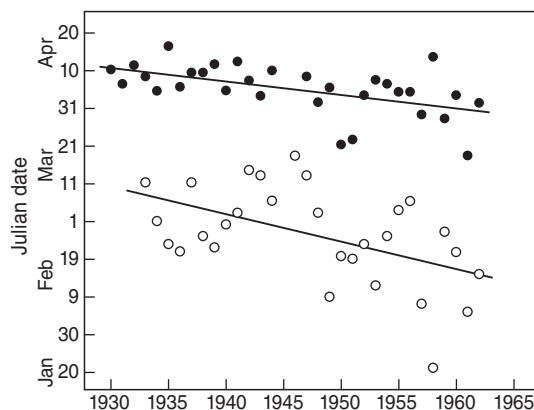


Figure 3.5: The advance of the time the first egg was found (filled circles) and the first black-tailed godwit arrived (open circles) in the province of Friesland, The Netherlands, from 1930 until 1962. First egg: $F_{1,29} = 11.83$; $R^2 = 0.29$; $P = 0.002$. Arrival: $F_{1,27} = 9.17$; $R^2 = 0.25$; $P = 0.005$. Data retrieved from Haverschmidt (1963).

which may be especially true for long-distance migrants (Miller-Rushing *et al.*, 2008). A similar argument has been given for why pied flycatchers *Ficedula hypoleuca* adjusted laying date insufficiently relative to the advancing food peak for their offspring (Both & Visser 2001). After advancing both laying and arrival date, similar constraints during the non-breeding season may have prevented godwits from further advancing arrival timing, and possibly also subsequent timing of breeding.

In contrast to most other bird species, we found that black-tailed godwits laid later in warmer years. Since 1976, warm Mays are correlated with warmer, and maybe drier winters, which may reduce food availability for adults pre-egg laying (Pearce-Higgins *et al.*, 2009), which could lead to females delaying breeding. This is unlikely since (1) we did not find relationships of timing of breeding with precipitation parameters during early spring, and (2) in the Dutch climate, warmer winters are wetter (correlation between January–February temperature and January–March rainfall, 1976–2007; $R = 0.55$, $P = 0.001$), which should increase food availability and hence advance timing of breeding (Both *et al.*, 2005). A possible explanation for the counter-intuitive positive correlation between May temperatures and breeding time is that it was caused by a bias towards late hatching dates due to more second clutches in warmer springs.

Instead of an advancement in timing of breeding, the effect of hatching date on hatchling mass became more negative over time (Fig. 3.4). Apparently, godwits invested more in early eggs and less in late eggs, leading to no change in mean annual hatchling mass. Investment in early eggs was highest in years when the temperature-sum 180°C was reached early (Fig. 3.4) – already from January to early March. Few godwits arrive on the breeding grounds before late February and they cannot use the weather before arrival as a direct cue for timing and investment decisions (Beintema *et al.*, 1995; van den Brink *et al.*, 2008). We suggest that in years with a lower temperature-sum 180°C – warm and wet winters with frost periods that were short and with fertilizer applications that were early (Kruk *et al.*, 1996) – lead to a high and early availability of earthworms (Jordan *et al.*, 2004; Timmerman *et al.*, 2006). After

warmer winters, returning female godwits may thus find it easier to achieve the required nutritional state to produce eggs.

Early born and heavy chicks have a higher survival probability than late born chicks (Roodbergen & Klok 2008; Schekkerman *et al.*, 2009). Thus, it would seem advantageous for females to invest less into late chicks, especially in years when mowing is early. We indeed find a steeper decline in hatchling mass in warm springs when mowing occurs earlier (Fig. 3.4F). But why would godwits be naturally prepared to invest less in late offspring during warmer years? It is possible that higher temperatures predict food shortages for late born chicks also in situations without mowing, because warmer weather is linked with an earlier peak in insect abundance (Nooker *et al.*, 2005; Tulp & Schekkerman 2008), and thus may mean that the food situation is less good later.

Long-lived birds are normally expected to be rather prudent in their current reproductive investment, because they still have a long future reproductive life (Drent & Daan 1980). They are expected to base their reproductive investment less on the needs of the offspring than on those of the adult at the time of egg laying (Erikstad *et al.*, 1998), and, during shortages, are expected to invest less, abandon a clutch or even refrain from laying at all (Erikstad *et al.*, 1998; Weimerskirch *et al.*, 2001). Agricultural changes and climate change have altered the temporal fitness landscape of black-tailed godwits in more than one way, improving conditions for females during egg laying and reducing the value of late born chicks. While black-tailed godwits are apparently not (any longer) able to respond with laying date, they can adjust egg size. We suggest that on a continuous gradient of either maximizing adult survival or offspring fitness, godwits tend to invest and time their reproduction towards maximizing adult survival.

Acknowledgements

We want to thank It Fryske Gea, Fûgelwacht Warkum, Petra de Goeij, Valentijn van den Brink and Freek Mandema for invaluable help in the field. Martin Hinsch provided inspiring discussions. Dick Visser provided the figures. This work was done under the license number DEC 4112B following the Dutch Animal Welfare Act Article 9. This work was financially supported by a set-up grant to TP from the University of Groningen, a grant to JS from the Schure-Beijerinck-Popping foundation and a grant by the Vogelbescherming Nederland. PL was supported by a grant from the Portuguese Science Foundation.

Four

Sexual dimorphism in plumage and size in black-tailed godwits *Limosa limosa limosa*

Julia Schroeder, Pedro M. Lourenço, Marco van der Velde,
Jos C.E.W. Hooijmeijer, Christiaan Both & Theunis Piersma

Abstract

Systematic sex-related differences in size and plumage are informative of sex-specific selection pressures. Here, we present an analysis of sexual dimorphism in body size and plumage of black-tailed godwits *Limosa limosa limosa* from a breeding population in The Netherlands. Molecular methods were used to unambiguously assign the sex of captured birds. To quantify breeding plumage, we developed nine plumage scores. These scores describe the intensity of orange in the breast plumage, the extent of black bars on the belly, the coverage and number of breeding feathers on the back, the conspicuousness of the white eye stripe, the extent of white plumage on the head, the percentage of orange color in the bill and the percentage of white and black spots covering the neck. Most females were structurally bigger, and had a greater body mass. Nonetheless, we found a greater overlap in bill length between the sexes than expected on the basis of literature data: biometrics alone are not sufficient to correctly discriminate between the sexes. Black-tailed godwits are sexually dimorphic mostly with respect to the amount of white spots on the neck, females being of lighter color than males. In addition, females showed fewer black bars and less orange on the breast, had more white in the head and lesser and a smaller extent of breeding feathers on the back. Interestingly, we found a genotypic polymorphism on the sex-related CHD1 gene on the Z chromosome, commonly used for molecular sexing in birds. Males of the less frequent genotype had significantly more white in their plumage and had fewer black bars on their breast, while in females no differences between the two genotypes were found.

Introduction

SEXUAL DIMORPHISM IS THE systematic phenotypic difference between females and males of the same species (Bennett & Owens, 2002). In birds, the most obvious and abundant differences between the sexes are body size and plumage coloration. The extent and variation of sexual dimorphism is correlated with variations in mating systems, sex differences in parental care and the frequency of extra-pair paternity (Darwin, 1871; Bateman, 1948; Trivers, 1972; Andersson, 1994; Owens *et al.*, 1995; Sandercock, 2001). In waders with aerial display, small males are assumed to be better performers because small body size is thought to enhance agility, which in turn positively influences fitness (Jönsson & Alerstam, 1990; Figuerola, 1999). Together with a fecundity advantage for bigger females (Summers & Underhill, 1991; Sandercock, 1998), selection should lead to a female-biased dimorphism in size in these species (Jönsson & Alerstam, 1990; Székely, Freckleton & Reynolds, 2004). It has been suggested that plumage quality informs potential mates about migratory condition, parasite resistance and age (Piersma & Jukema, 1993; Piersma *et al.*, 2001; Battley, 2007). If these plumage traits have a genetic basis, it can lead to selection towards the more competitive sex being more ornate. Plumage may also function as camouflage in waders and other cryptic ground breeders (Heinsohn, Leffe & Endler, 2005; Hill & McGraw, 2006b). Thus, the sex that spends more time incubating often shows a more cryptic plumage. These mechanisms are expected to go hand in hand and can lead to a strong plumage dimorphism.

Black-tailed godwits *Limosa limosa limosa* are one of the stronger sexually size-dimorphic wader species and show some degree of plumage dimorphism. Males are the smaller and the more colorful sex (Glutz von Blotzheim, Bauer & Bezzel, 1985). Male nuptial display includes acrobatic and fast flights (Lind 1961). Pairs typically stay together for several years and both partners incubate the eggs and care for the young equally (Beintema *et al.*, 1995). Two conditions are crucial to study sexual size and plumage dimorphism: (1) a reliable method to unambiguously identify sex independent of plumage and size characters; and (2) repeatable measurements describing size and plumage. When birds cannot be sacrificed and dissected, non-molecular sexing and studying sexual dimorphism always includes some degree of circular logic. Up to now, all studies reporting differences between sexes in black-tailed godwits of the nominate race *limosa* either used dissection and sex determination by gonads (Glutz von Blotzheim *et al.*, 1985), discriminant function analysis (Groen & Yurlov, 1999; Glutz von Blotzheim *et al.*, 1985) or behavioral traits, size and plumage together (Groen, 1993; Groen & Hemerik, 2002) to sex adult birds. Gunnarsson *et al.* (2006a) used molecular techniques to sex adult Icelandic black-tailed godwits *Limosa l. islandica*, a closely related subspecies.

In this paper, we report on the degree of sexual dimorphism in black-tailed godwits with respect to size measurements, body mass and plumage traits. In contrast to size measurements, the description and quantification of plumage is far from standardized in birds (Hill & McGraw, 2006a). Here we defined nine plumage scores and test them

for observer repeatability. To study sexual dimorphism, birds were sexed genetically and we then examined correlations between size and plumage variables and between the sexes. We tested for between year repeatability of all traits.

Methods

Study area

Our study area (52°59'N, 5°24'E) in the province of Friesland, The Netherlands, consists of 300 ha extensively managed meadows and an adjacent wetland along the shore of the Lake IJsselmeer. This area, called Workumerwaard, is divided into two parts by a summer dike alongside Lake IJsselmeer. The inner part of about 215 hectares consists of 22 meadows separated by water-filled ditches. A paved road intersects the area. The meadows are managed according to agricultural nature management plans, which encompass mowing only after 8 or 15 June and no use of artificial fertilizer. Management is done by the provincial nature conservation organization 'It Fryske Gea' and by farmers. The outside part is a nature reserve and managed by It Fryske Gea. Entry is not allowed and mowing is limited to the summer dike. The area is not fertilized, but in summer cows and horses graze it in low density.

This study took place during the breeding season of godwits in March until June in the years 2004–2006. Every year, local volunteers of the meadowbird conservation society 'Fûgelwacht Warkum' searched the area thoroughly for godwit nests and reported approximate locations to us. We revisited the nests and determined exact positions with handheld Garmin GPS 12 to the nearest 2 m. Catching was scheduled only three days before the hatching day. The hatching date of a clutch was estimated by measuring the degree of buoyancy of the eggs which is related to the incubation stage (van Paassen *et al.*, 1984; Beintema *et al.*, 1995). When cracks were found in the eggs three days before the estimated hatching date, or when the chicks were audibly beeping from inside the eggs, catching attempts were undertaken with either a walk-in trap or an automatic fall-trap. Once a bird entered a walk-in trap and sat down on its nest, an observer started running towards it causing the bird to flee. The funnel shaped entrance prevented the bird from escape. The automatic fall-trap consists of two metal rings connected with mistnet fabric. Both rings rest on three metal poles that are placed around a nest. This construction allowed a bird to enter the trap from all sides. Once the bird sat down on its nest, the lower ring was released by a remote control and the bird was trapped. The two types of traps worked well though some individuals were easier to catch than others. We never observed nest abandonment after catching attempts.

Body size

Within 15 min after capture, birds were weighed to the nearest gram. The following body size dimensions were also measured: wing length (flattened and straightened, ± 1 mm), bill length (exposed culmen, ± 0.1 mm), total head length (± 1 mm), tarsus

length (± 0.1 mm), and tarsus + toe length (tarsus plus mid-toe length without nail ± 1 mm). We measured and weighed 70 female and 64 male black-tailed godwits.

Plumage

To quantify plumage, we took digital pictures of each bird with a resolution of 2272×1704 pixels. Photos were taken with Nikon Cool Pix 4500 cameras of the back, the breast and the head in profile (Fig. 4.1). This happened after the bird was color-ringed so that it could be identified individually on the photo. For objective color judgment, we added a grey card to every picture of the front part of the birds (Fig. 4.1).

We scored nine plumage variables (Table 4.1). The bars score describes the extent of black bars on the belly on a scale from one to five. Orange score is the intensity of orange a bird displays on the breast. Orange bill is the percentage of orange coloration in the bill in relation to the total bill length, with an accuracy of five percent. Eye stripe is the extent and intensity of the white eye stripe, on a scale from one to five. White in head is the percentage of white feathers covering the head in profile, with an accuracy of five percent. White spots is the percentage of the neck covered with white feathers, with an accuracy of ten percent. The black spots score is the percentage of the neck covered with black spots with ten percent accuracy.

Godwits, like many other waders, only partially moult into breeding plumage. This is most clearly visible on the back of a bird, where between few to all feathers can be moulted into breeding feathers. It is not known whether the remaining feathers are also moulted into a winter version, or not at all (Battley *et al.*, 2004). Back score is the extent of breeding feathers covering the back of a bird, on a scale from one to five.

Table 4.1: Descriptions and range of plumage scores for black-tailed godwits. See text for further explanations of these scores.

| Plumage score | Range | Description |
|-------------------|-------|--|
| Bars | 1–5 | 1= no black bars on breast and belly, 2= some, 3= normal extent of black bars, 4= until the legs, 5= black bars extend the legs and underneath the tail |
| Orange | 1–5 | 1= winter plumage, 2= weak orange, 3= normal orange, 4 = dark orange-red, 5 = very dark red, like L.l. islandica on the breast and neck |
| Bill | 0–100 | percentage of orange in bill in relation to total bill |
| Eye stripe | 1–5 | 1= no eye stripe, 2= barely visible, 3= normal extent from ear to bill, 4= broad, 5= very broad and long |
| White head | 0–100 | percentage of white in head |
| White spots | 0–100 | percentage of neck covered in white feathers |
| Black spots | 0–100 | percentage of neck covered in black feathers |
| Back | 1–5 | 1= no breeding feathers on the back, 2= some breeding feathers, but less than 1/3 of the back covered, 3= 1/3 to 2/3 of the back covered, 4= more than 2/3 of the back covered, 5=total back covered |
| Breeding feathers | count | absolute number of breeding feathers on the back |



Figure 4.1: Examples of the photos used to score breeding plumage of black-tailed godwits. Top row: ventral photo showing exemplary variation in bars score, orange score and white and black spots. Middle row: side views of godwits, used to score white head, eye stripe and orange bill score. Bottom row: back with extracted wing to score back score and count breeding feathers on back. Females: top left, middle right, bottom left.

Finally, the absolute number of breeding feathers on the back defines the variable breeding feathers; they were counted per single feather.

We had complete data on breeding plumage scores of 57 female and 53 male godwits. These numbers were lower than the ones used for size dimorphism analysis because we only used photos on which birds could be scored unambiguously. To test for within-observer repeatability, JS scored photos twice with the identity of birds unknown to her. Repeatabilities were calculated following Lessells & Boag (1987), standard errors as described by Becker (1984). Observer repeatability was high; the lowest repeatabilities were 0.80 for orange score, 0.84 for eye stripe and 0.87 for orange in bill. All other plumage scores reached repeatabilities >0.90 . Standard errors were low (≤ 0.08).

Molecular sexing

A blood sample of 20 μl was taken from the brachial wing vein before body size and plumage measurements were taken. The area around the vein was cleaned with a cotton ball dipped in ethanol. The blood was drawn from the puncture with a sterilized microcapillary tube. The sample was stored in 96% ethanol at -20°C for the first weeks and at -80°C thereafter. DNA was extracted in the laboratory using the chelex extraction method of Walsh, Metzger & Higuchi (1991). Birds were sexed following Griffiths *et al.* (1998). This method is based on the amplification of a supposedly neutral fragment of an intron on the conservative CHD1 gene located on the sex chromosomes. These fragments differ in base pair length between the Z and the W chromosome in most bird species. Males with ZZ genotype have two fragments of the same length, whereas females of the genotype ZW have two fragments of unequal length. Fluorescently labelled PCR products were separated on an ABI 377 automatic sequencer. Subsequently their length was determined using Genescan 3.1 software.

We observed a polymorphism on the Z chromosome; PCR products originating from this chromosome were either 374 or 378 basepairs in length. The PCR product of the W chromosome was 393 basepairs long. Birds with genotypes 374/378 basepairs and 378/378 basepairs were scored as males (genotype 374/374 basepairs was not observed) and birds with genotypes 374/393 basepairs and 378/393 basepairs were scored as females. To verify our results and as recommended by Dawson *et al.* (2001), we also used the method of Fridolfsson & Ellegren (1999), which consists of amplifying a different fragment of an intron on the same gene. These PCR products were separated on a 3.3% agarose gel. These fragments are also of different length on the Z and W chromosome. This confirmed our previous sex assignment and we did not find a polymorphism.

Statistics

To study how the different variables are correlated with each other with respect to sex, we entered standardized values of body mass and all size variables in a first analysis of principal components (PCA) and all plumage variables in a second PCA. The first two principle components with eigenvalues bigger than one were extracted. We plotted the

score of the first (PC1) and second principal component (PC2) of each bird in a bico-ordinate system with PC1 and PC2 as axes (Gabriel 1971; see Battley *et al.*, 2001 for an example). Additionally, the eigenvalue loadings of each variable were plotted in the same graph as a vector. The length and direction of these vectors reveal correlations between different variables. The smaller the angle between two vectors, the more both vectors correlate with each other. A longer vector indicates a better fit. The positions of males and females in this plot relative to the vectors indicate the strength and direction of the dimorphism for the respective variables. Individuals might show trait variation between years. We calculated individual repeatabilities between years separately for females and males. We additionally tested for differences in size and plumage in birds with the less frequent allele with 374 basepairs for both sexes. We used Statistica 7.0 for Microsoft Windows XP, SPSS 14.0 and R 1.14 for Mac OS X to calculate statistics.

Results

Sexual dimorphism

All morphological variables, as well as body mass, showed a bimodal distribution (Fig. 4.2). Female godwits were bigger and heavier than males, with all variables significantly different between the sexes (Table 4.2). Wing length was the least dimorphic trait; the most dimorphic traits were body mass and bill length (Fig. 4.3). However, there was considerable overlap. The distributions of female body mass and size were skewed to the left; a few females were as small as males.

The distributions of the plumage scores were less distinctly bimodal. Female and male godwits differed significantly in orange score, white spots, back score, breeding feathers, bars score and white in head (Table 4.3). They did not differ with respect to bill, black spots and eye stripe score. The biggest sexual dimorphism between males and females was found in white spots and in white head (Fig. 4.3). Males were more

Table 4.2: Sexual size dimorphism in the black-tailed godwit. Depicted are means with standard deviations and results of separate two-sided *t*-tests testing for differences between sexes, with *P*-values. Body mass in grams, lengths in mm.

| Size variable | Females <i>n</i> = 70 | | | Males <i>n</i> = 64 | | | <i>t</i> | <i>P</i> |
|---------------|-----------------------|-------|---------|---------------------|-------|---------|----------|----------|
| | Mean | SD | Range | Mean | SD | Range | | |
| Body mass | 305.50 | 21.15 | 231–354 | 254.88 | 17.70 | 219–304 | 14.95 | <0.001 |
| Wing | 224.13 | 6.75 | 208–241 | 213.08 | 5.31 | 199–227 | 10.47 | <0.001 |
| Bill | 105.04 | 6.09 | 86–117 | 89.77 | 5.51 | 81–102 | 15.17 | <0.001 |
| Total head | 145.6 | 7.15 | 122–159 | 128.59 | 6.05 | 112–144 | 14.80 | <0.001 |
| Tarsus | 80.38 | 3.93 | 72–89 | 73.33 | 3.35 | 66–81 | 11.14 | <0.001 |
| Tarsus toe | 125.31 | 5.82 | 109–142 | 115.86 | 4.09 | 106–127 | 10.80 | <0.001 |

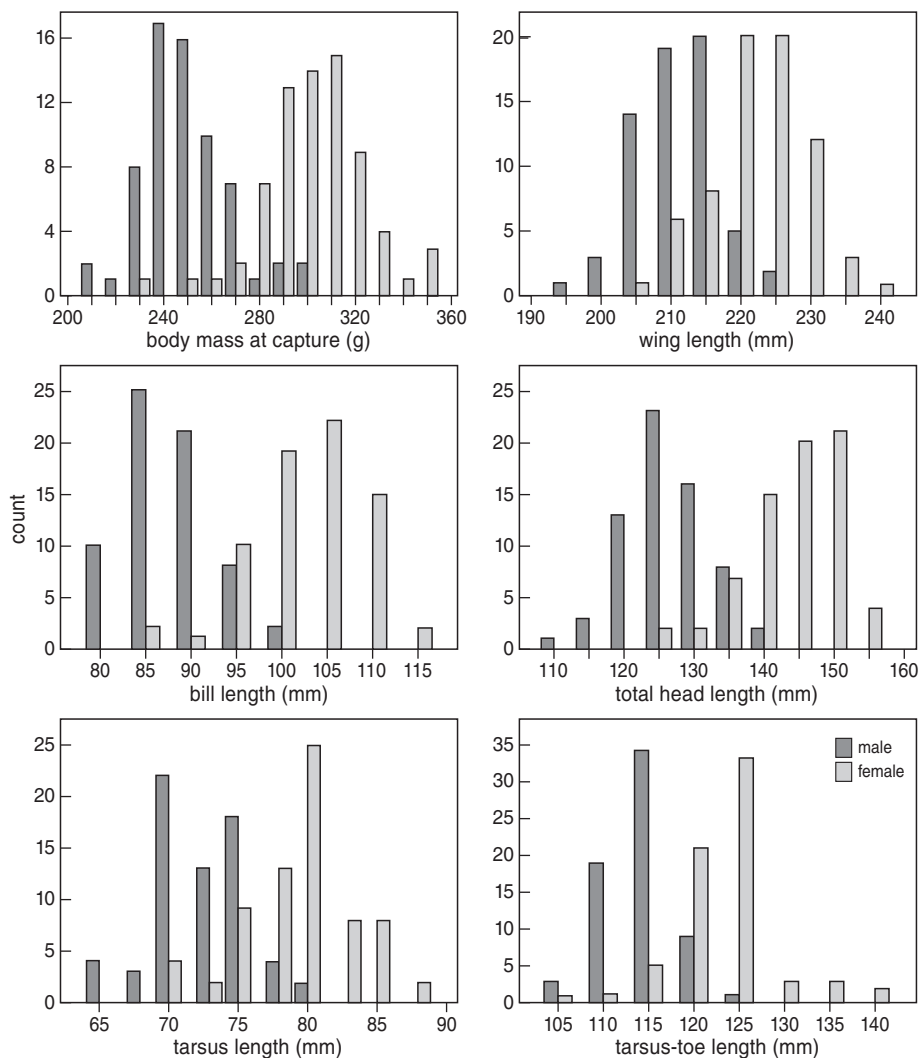


Figure 4.2: Histogram of body mass, wing length, bill length, total head length, tarsus length and tarsus-toe length in female and male.

orange and were more ornate than females and had fewer white in their plumage. The dimorphism with respect to white spots was more than seven times as pronounced as that of any other variable (Fig. 4.3).

The first two principle components of a PCA explained 86% of the variation in morphological variables and body mass (PCA: $KMO = 0.85$, $\chi^2 = 873.94$, $P < 0.001$). To examine the correlation structure of the morphological variables, we produced a biplot figure (Fig. 4.4). Bill length and total head length were correlated with each other, as were tarsus length and tarsus-toe length. Wing length was slightly stronger correlated with bill length and total head length than body mass. However, body mass

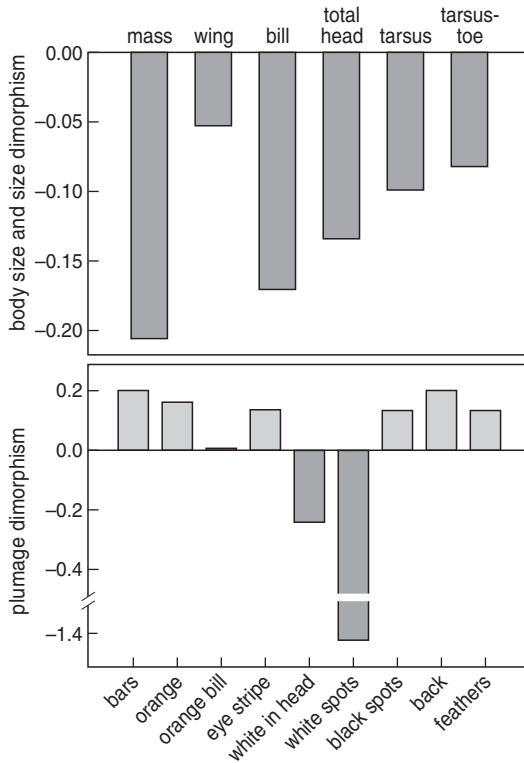


Figure 4.3: Sexual dimorphism of size and mass measurements (top) and plumage variables (bottom). Positive values indicate a male-biased dimorphism whereas negative values indicate females-biased dimorphism on the respective variable. N.s. indicates non-significant differences between females and males (statistics see Table 4.2 and 4.3).

Table 4.3: Sexual plumage dimorphism in the black-tailed godwit. Depicted are means with standard deviations and results of separate Mann-Whitney- U tests testing for differences between sexes, with P -values.

| Size variable | Females $n = 70$ | | | Males $n = 64$ | | | t | P |
|-------------------|------------------|-------|-------|----------------|-------|-------|-------|--------|
| | Mean | SD | Range | Mean | SD | Range | | |
| Bars | 3.18 | 1.20 | 1–5 | 3.89 | 1.09 | 1–5 | –3.11 | 0.02 |
| Orange | 3.36 | 0.78 | 2–5 | 4.06 | 0.86 | 2–5 | –4.10 | <0.001 |
| Bill | 66.00 | 9.17 | 50–80 | 66.20 | 7.97 | 50–80 | –0.16 | 0.87 |
| Eye stripe | 2.88 | 1.20 | 1–5 | 3.23 | 1.36 | 1–5 | –1.39 | 0.16 |
| White head | 46.23 | 24.66 | 5–90 | 35.75 | 26.26 | 0–90 | –2.16 | 0.03 |
| White spots | 33.33 | 24.66 | 5–90 | 13.11 | 21.24 | 0–90 | –5.03 | <0.001 |
| Black spots | 9.47 | 11.98 | 0–50 | 8.83 | 12.11 | 0–50 | –0.25 | 0.80 |
| Back | 2.66 | 0.97 | 1–5 | 3.34 | 1.04 | 1–5 | –3.54 | <0.001 |
| Breeding feathers | 23.40 | 11.48 | 2–52 | 28.4 | 12.30 | 2–54 | –2.36 | 0.02 |

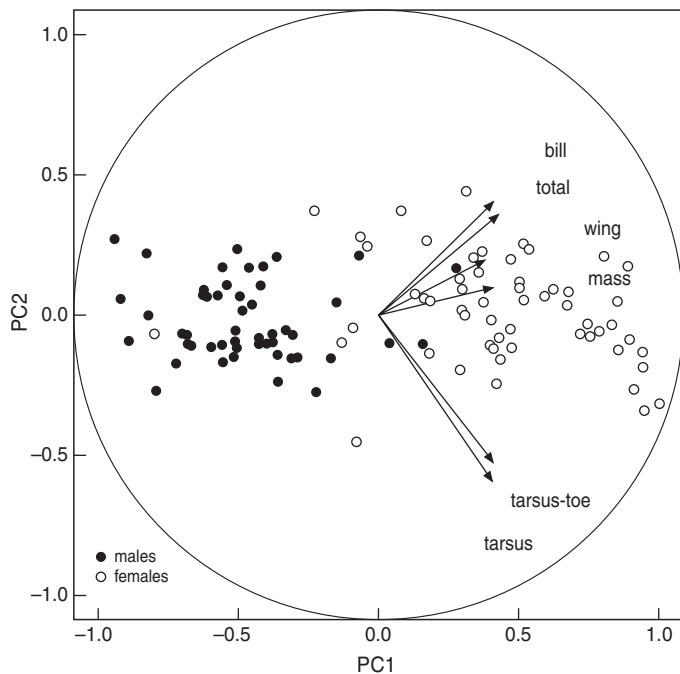


Figure 4.4: Relationships between biometric variables (wing length, tarsus length, tarsus-toe length, bill length, total head length) and body mass. The axes represent the first two principal components of the standardized data for all birds. Arrows represent the loadings of each variable on the first two components. Bill and total head length are strongly correlated with each other, as are tarsus and tarsus-toe length.

was least correlated with any of the size traits, confirming that body mass is best seen as an index of mass-corrected storage (van der Meer & Piersma, 1994).

The first two principle components of a PCA of plumage traits explained 57% of the variation in the variables (PCA: $KMO = 0.75$, $\chi^2 = 355.35$, $P < 0.001$). A close inspection of the biplot figure for the plumage variables (Fig. 4.5) revealed a strong correlation between the plumage traits bars score, orange score, back score and breeding feathers. The amount of white spots was negatively correlated with these. Further, eye stripe score was negatively correlated with bill score, and to a lesser extent with black spots. The score white in head was not correlated with any of the other plumage traits.

Between-year variation

For both females and males that were recaptured in more than one year, size measurements were highly repeatable (Fig. 4.6). The only exception was tarsus length in females (0.41), which was due to one bird that had a tarsus length of 83.2 and 88.8 mm in two subsequent years, while tarsus-toe length stayed the same (128 mm), suggesting a measurement error. Body mass was highly repeatable in males between

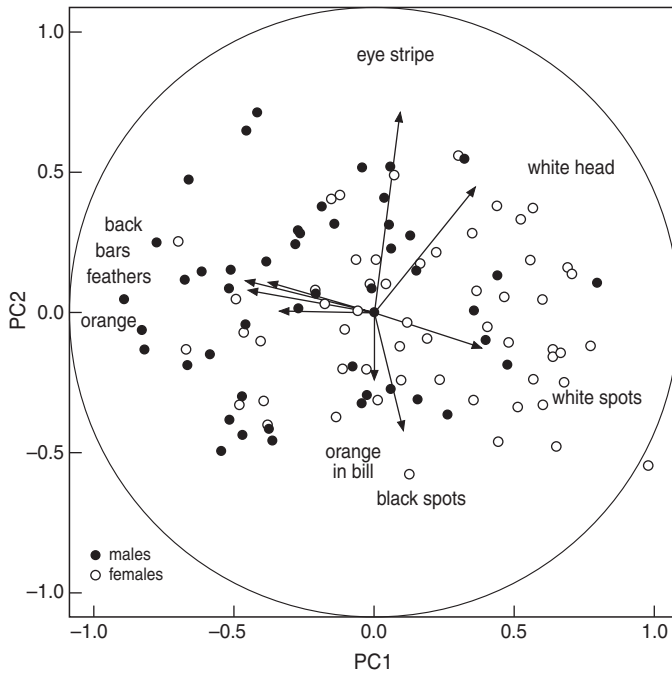


Figure 4.5: Relationships between the plumage variables (eye stripe, white in head, white spots, black spots, orange in bill, orange, bars and breeding feathers on back). The axes represent the first two principal components of a principal component analysis on the standardized data for all birds. Arrows represent the loadings of each variable on the first two components.

years (0.72), but not in females (−0.32). This was not due to a single outlier; the mean mass difference of a female between two consecutive years was 38 ± 23 g, whereas in males it was 7 ± 7 g (averages \pm SD).

For females, orange in bill, white in head and white spots were most repeatable between years (0.82, 0.84, 0.79, respectively), whereas bars score, orange score and black spots were least repeatable (0.08, 0.34 and 0.37 respectively). In males, bars score, eye stripe, white head, back score and breeding feathers showed repeatabilities of >0.70 , and bill >0.50 . Orange score and black spots showed low repeatabilities, but in contrast to females, white spots was least repeatable.

Genotype correlated plumage variation

We discovered a genotypic polymorphism on the Z chromosome when using P2P8 primers (Griffiths *et al.*, 1998) for molecular sexing: 29% of all males had the less frequent 374/378 genotype. Nine percent of female godwits carried the 374/393 genotype, while all others had the 378/393 genotype. The frequencies did not deviate from Hardy-Weinberg equilibrium. We tested for differences in size and plumage between birds with and without the less frequent 374 basepair allele. We found no difference in

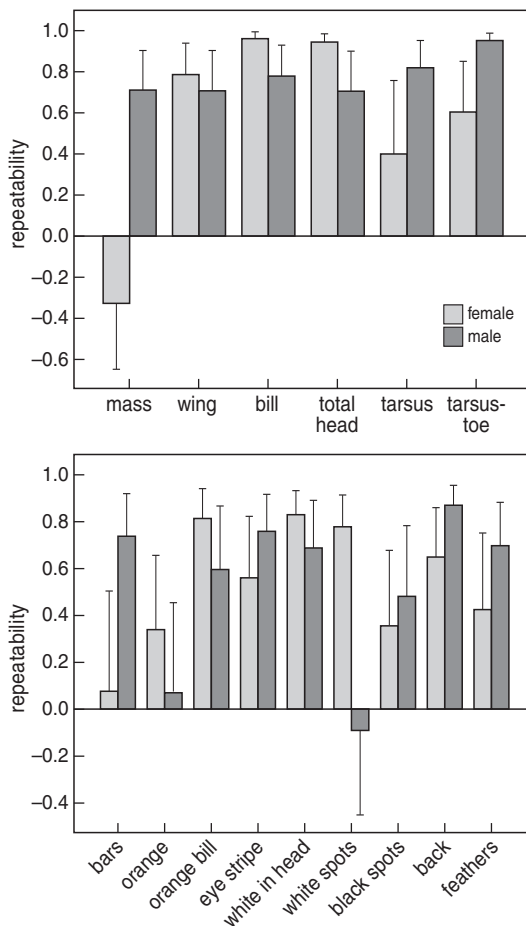


Figure 4.6: Repeatabilities within individual black-tailed godwits between years (light grey = females, dark grey = males). Error bars indicate standard errors. Top: repeatability of mass and size measurements. $n = 6$ females and 7 males. Bottom: repeatability of plumage scores taken from pictures. $n = 6$ females and 7 males.

size, body mass and plumage in females. In males, there was no difference in size and mass, but males with the genotype 374/378 scored higher on whiter spots (Mann-Whitney $U = 2.53$, $P = 0.012$, $n_{374/378} = 19$, $n_{378/378} = 20$) and lower on bars (Mann-Whitney $U = -2.69$, $P = 0.0072$, $n_{374/378} = 18$, $n_{378/378} = 19$) than males with the more common 378/378 genotype.

Discussion

Sexual dimorphism

Male black-tailed godwits were confirmed to be smaller and lighter than females. Our mean values of body mass did not differ significantly from the averages presented by Glutz von Blotzheim *et al.* (1985) nor from Groen & Yurlov (1999) or Cramp & Simmons (1983), with data from godwits breeding in the same geographic range as our population, in Western Europe. However, in our population, we found three

females with a bill smaller than the lowest given by Groen & Yurlow (1999), and even 10 females with a bill shorter than given in Glutz von Blotzheim *et al.* (1985). Several of our females had bills up to one cm shorter than the shortest bill length cited in this literature. Yet, sexing was unambiguous. The two female birds with the shortest bills were caught in two years and DNA-samples of both years confirmed female sex. Furthermore, both birds were paired to DNA-confirmed males. It is likely that the relatively small sample size ($n = 20$) of Glutz von Blotzheim *et al.* (1985) prevented them from detecting these females with a short bill length. Similarly, the discriminant function analysis used by Groen & Yurlow (1999) was probably not able to distinguish these females from males. All our variables, including the most dimorphic traits, showed large overlaps between males and females (Fig. 4.2, Table 4.2, Table 4.3). We therefore like to stress the importance of reliable molecular assays to unambiguously assign sex also in dimorphic species.

We established that female godwits have less black bars on the belly, a lower orange intensity, display more white in their head and neck and have fewer breeding feathers on their back. This is consistent with verbal descriptive reports in the literature (Cramp & Simmons, 1983; Glutz von Blotzheim *et al.*, 1985; Gunnarsson *et al.*, 2006a). We also found that the plumage traits back score, bars, breeding feathers, orange score and white spots were correlated with each other; a bird that scored high on one variable also scored high on the other, and low on white on neck. This indicates that colorful individuals are colorful with respect to all these traits. Further, the distinct sexual dimorphism on both size and plumage traits indicates some degree of sexual selection pressures on these traits.

Between-year variation

Between-year repeatability was relatively high for size measurements in males as well as in females. However, body mass was not repeatable in females, but it was in males. Female godwits may be able to vary their strategic nutrient energy stores responding to external variation and temporal needs.

For different plumage scores, individual repeatability between years differed considerably between the sexes. For both sexes, orange in bill, eye stripe, white in head, back score and breeding feathers were repeatable between years, whereas orange score and black spots were not. This may give hints as to the functions of the various plumage traits. In birds, multiple ornaments may communicate multiple messages (McGraw & Hill, 2000; McGraw *et al.*, 2002; Doucet & Montgomerie, 2003). Traits that are not repeatable, but phenotypically plastic, may reflect a bird's state with regard to variable condition, mediated by environmental variability. Such plumage traits may be a signal for body condition with respect to body mass, health status or parasite load (Piersma & Jukema, 1993; Piersma *et al.*, 2001). Senar & Quesada (2006) propose that these characters may play a role in sexual selection, too. In spite of a low repeatability of environmentally influenced traits, the relative ranks of the individuals could be maintained if the variation in the environment would be the same for all individuals.

There were marked differences between the sexes. The percentage of white spots on the neck was highly repeatable in females but not in males. It was the other way around in the bars score. These differences appear in the direction of the sexual dimorphism of the traits. These may be plumage traits that play an important role in sexual signalling or reflect differences in natural selection pressure between sexes. One sex might be able to afford plasticity in some traits but not in others (Piersma & Drent, 2003). Sample size prevented us from testing for temporal trends within one season. However, as we caught all birds on their nest, plumages have consistently been scored at a time when moult must have been complete.

Genotype correlated plumage variation

We detected a polymorphism on the location used for unambiguous sexing, and found correlated variation in plumage traits of males. A similar polymorphism on the same gene occurs in four auklet species (*Aethia pygmaea*, *A. pusilla*, *A. cristatella*, *Cyclorhynchus psittacula*, Dawson *et al.*, 2001), Moorhens *Gallinula chloropus* (Lee *et al.*, 2002), Red Knots *Calidris canutus* (A.J. Baker, pers. comm.) and Upland Sandpipers *Bartramia longicauda* (B.A. Sandercock, pers. comm.). Lee *et al.*, (2002) found a reduced survival for Moorhen males with the rarer genotype and B.A. Sandercock (pers. comm.) found a reduced reproduction in males with the rarer genotype. These findings are consistent with the idea that these plumage ornaments have a genetic basis. The primer set we used primes for a fragment of an intron of the CHD1 gene (Lee *et al.*, 2002). CHD1 was proposed to be a regulatory gene and therefore thought to be conservative (Lee *et al.*, 2002). Black-tailed godwits are now the third species in which variation in this region is correlated to functional traits.

On agarose gels the genotypes 374/378, 374/393 & 378/393 all showed two distinct bands, but unfortunately it was impossible to discriminate between the three genotypes accurately. Consequently, males with the 374/378 genotype can be misinterpreted as females. In this study 29% of all males could have been missexed if only using agarose gels, as is widely practiced in molecular sexing of birds for studies in evolutionary ecology. Our study shows that especially in species with an overlap in body dimensions between both sexes, neither standard molecular protocols nor sex assignment based on body dimensions give constantly correct results. Molecular sex assignment should always be verified in future studies of topics related to sexual differences and sex ratios.

Acknowledgements

We want to thank It Fryske Gea, Fûgelwacht Warkum, Niko Groen, Petra de Goeij, Valentijn van den Brink, Rosemarie Kentie and Freek Mandema for invaluable help in the field and the anonymous referees for their comments on the manuscript. This work was done under the license number DEC 4112B following the Dutch Animal Welfare Act Article 9. This work was financially supported by a set-up grant to TP from the University of Groningen, a grant to JS from the Schure-Beijerinck-Popping foundation and by the Vogelbescherming Nederland. PL was supported by a grant from the Portuguese Science Foundation.

Five

A possible case of contemporary selection leading to a decrease in sexual plumage dimorphism in a grassland-breeding shorebird

Julia Schroeder, Pedro M. Lourenço, Jos C.E.W. Hooijmeijer,
Christiaan Both & Theunis Piersma

Abstract

In sexually dimorphic species, males with more exaggerated plumage ornamentation generally have higher body condition, are preferred by females, and have higher reproductive output. In contrast to the majority of studies, we describe that less ornamented males of the monogamous and sexually dimorphic black-tailed godwit *Limosa limosa* were larger and heavier during late incubation than more ornamented males, and were mated with females that laid larger eggs. In females we detected no such correlations. If paler and larger males indeed have higher fitness, this suggests ongoing selection for a reduction in male ornamentation, leading to a decrease in plumage ornamentation. We found evidence for such a change since 1840: male ornamentation in museum specimens became progressively less in all our measured plumage traits. One explanation for this could be that formerly sexually selected plumage traits are now selected against, and that the sexual plumage dimorphism of West-European breeding black-tailed godwits might be waning. Concomitant with this change in plumage coloration, this species has experienced enormous human-mediated changes in both breeding and non-breeding habitat, and we suggest that these might have led to increased costs or reduced fitness benefits for highly ornamented male godwits.

Introduction

INDIVIDUAL BIRDS MAY SIGNAL their reproductive qualities to prospective mates by conspicuous plumage coloration (Hill & McGraw, 2006b). Such ornamentation must be costly to be reliable as honest signals of the qualities of potential mates (Zahavi, 1975). Indeed, many studies have found that the plumage ornamentation of males is positively correlated with body condition variables, female preference and reproductive success (for overviews see Andersson, 1994; Hill, 2002; Hill & McGraw, 2006b). If such selection pressures are less pronounced for females, this should lead to sexual plumage dimorphism (Andersson, 1994; Ryan, 1997). The degree of sexual dimorphism thus represents the outcome of the opposing pressures of natural and sexual selection (Darwin, 1871), with natural selection representing the costs of a trait, and sexual selection the benefits. However, costs and benefits may change over time, varying with environmental changes, and this may lead to sexually selected extravagant traits receding or even getting lost (Wiens, 2001). Loss of sexually selected traits can be due to either variation in gene frequencies caused by directional selection, or phenotypes changing flexibly in response to environmental changes. Directional changes in sexually selected phenotypes in populations have been rarely observed (for an example see Phillips & Furness, 1998), and few studies of sexually dimorphic bird species have shown that less ornamented males have higher fitness (but see Sætre *et al.*, 1997; Griffith *et al.*, 1999).

The aim of this study is twofold. (1) Present a correlational analysis of sexually selected plumage coloration, body size, body condition and female reproductive investment (egg volume) in the sexually dimorphic black-tailed godwit *Limosa limosa*, breeding in The Netherlands. As we find that male black-tailed godwits with less colorful and more female-like plumage were larger, in better condition and paired to females laying larger eggs than more colorful godwits, we set out (2) to find circumstantial support for any changes in male plumage coloration over the last one and a half century. To do so we examined historical museum skins over the time span between 1840 and 2007 for changes in plumage ornamentation and size.

Methods

Study species

Black-tailed godwits are ground-breeding waders of temperate areas in Europe and west-Asia (Beintema *et al.*, 1995). The nominate subspecies *L. l. limosa* migrates to West Africa in late summer. From December, they stage in rice fields on the Iberian Peninsula for 2–3 months (Lourenço & Piersma, 2008a) where they undergo pre-alternate molt. Black-tailed godwits are socially monogamous (Cramp & Simmons, 1983; Beintema *et al.*, 1995). Similar Charadrii species show low to no extra-pair paternity (Wallander *et al.*, 2001; Blomqvist *et al.*, 2002). Both parents incubate the clutch of four eggs in a nest scrape in open grassland. The chicks are precocial and nidifugous,

foraging mainly on above ground invertebrates until fledging (Beintema *et al.*, 1995; Schekkerman & Beintema, 2007).

Black-tailed godwits are sexual dimorphic with respect to body size and plumage: females are larger and have a paler alternate plumage than males (Cramp & Simmons, 1983; Groen & Yurlov, 1999; Gunnarsson *et al.*, 2006; Schroeder *et al.*, 2008a). Males have more black bars on their belly, more intense orange coloration on neck and breast, more breeding feathers on their back and less white in their head and neck plumage than female godwits (Schroeder *et al.*, 2008a). The orange and black plumage coloration of godwits is melanin-based (Toral *et al.*, 2008). Recent studies indicate that melanin plumage ornamentation can be physiologically and energetically costly (Jawor & Breitwisch, 2003; Niecke *et al.*, 2003; Griffith *et al.*, 2006) and may therefore represent an honest signal of male quality.

Study site and general methods

We did the contemporary part of this study in 2004 – 2007 in the Workumerwaard, Friesland, The Netherlands (52°59'N, 5°24'E), which consists of 214 ha of grassland used for silage-making and of 39 ha of freshwater marshland (Schroeder *et al.*, 2008; van den Brink *et al.*, 2008). We caught adult birds at 132 nests, and these nests were monitored from the beginning of the incubation period and we measured the length and width of eggs. We calculated the date of hatching by estimating the incubation stage by the inclination of floating eggs in water (van Paassen *et al.*, 1984; Liebezeit *et al.*, 2007).

We caught 80 female and 78 male birds with walk-in traps on the nests shortly before the estimated hatching date. We weighed the birds to the nearest g and the following body dimensions were measured: wing length (flattened and straightened, ± 1 mm), bill length (exposed culmen, ± 0.1 mm) and tarsus length (± 0.1 mm). To measure plumage characteristics, we took digital pictures of each bird with Nikon Cool Pix 4500 digital cameras. We took photos of the backside, the breast and the head in side view (Schroeder *et al.*, 2008a). A blood sample of 20 μ l was taken from the brachial wing vein and stored in 96% ethanol for genetic sex assignment. We used data taken at first capture or the most complete data set of each individual, this resulted in plumage data on 74 female and 72 male godwits of 122 nests (of 24 nests both partners were caught, and of 19 of those nests all data of both parents were known).

Fitness measures are difficult to obtain in this study system because fledglings were not detectable in the high vegetation of their preferred habitat, hence fledging success could not be measured for individual birds. Instead, we used average egg volume per nest as a measure of female reproductive investment. Variation in female reproductive investment is mostly in egg volume because black-tailed godwits have a relatively invariant clutch size of four eggs. Accounting for records of incomplete nests during the laying phase and partial egg loss during late incubation, more than 90% of all our nests contained four eggs at some point in time. Due to the high occurrence of partial egg loss (we recorded partial egg loss due to predation in >25% of all nests), we were

unable to record variation in original clutch size, and therefore we did not use clutch size in our analyses.

Egg volume is likely an important reproductive trait in this species, because it is directly related to chick mass at hatching (own data). The precocial chicks are vulnerable to adverse weather conditions and nutritionally challenging periods especially during the first few days of their lives (Beintema & Visser, 1988a; Schekkerman & Visser, 2001). Energy stores at hatching increase chick survival (own data; Beintema & Visser, 1988b), while most other factors responsible for low fledging success are of stochastic nature: agricultural activities, adverse weather conditions and predation (Gill *et al.*, 2007; Schekkerman & Beintema, 2007; Schekkerman *et al.*, 2008). Apart from pelvic bone size, egg volume is mainly affected by female nutritional status at laying (Amat *et al.*, 2001; Sanchez-Lafuente, 2004; Karell *et al.*, 2008). Female godwits that lay relatively bigger eggs are heavier and are likely of better quality than females that lay relatively small eggs (this study) and thus, egg volume may indicate female quality (van de Pol *et al.*, 2006). Females may invest more in reproduction if mated with a high quality mate (Cunningham & Russell, 2000; Loyau *et al.*, 2007). Thus, short of a better fitness measure, we considered average egg volume per nest to indicate female reproductive investment.

Molecular sex assignment

DNA was extracted by the chelex extraction (Walsh *et al.*, 1991). We sexed birds following Griffiths *et al.* (1998), but instead of using an agarose gel, we separated the fluorescently labeled PCR products on an ABI 377 automatic sequencer and determined their exact base-pair length with Genescan 3.1 software (Schroeder *et al.*, 2008a). We used this method because black-tailed godwits exhibit a genetic polymorphism on the Z-amplicon of Griffiths *et al.* (1998), which makes this method in this species prone to sexing errors (Dawson *et al.* 2001; Schroeder *et al.*, 2008a). Our results have, with the exception of the birds caught in 2007, been verified with the method of Fridolfsson & Ellegren (1999; Schroeder *et al.*, 2008a).

Historic data

We used museum skins collected in breeding areas in The Netherlands and Denmark (51°12'–57°44'N and 03°33'–12°44'E) of adult black-tailed godwits of the nominate race in alternate plumage only (Table 5.1). We excluded all museum skins collected before 1 April and after 31 June. Curators sexed birds on dissection by the presence of male or female internal sexual organs. Ambiguously or unsexed skins were excluded from the analysis. We took dorsal and ventral photos of the skins under standardized conditions. In total, we scored plumage of 61 female and 79 male black-tailed godwit skins from 1840–1997 (Fig. 5.1, Table 5.1). We measured wing length (flattened and straightened, ± 1 mm), bill length (exposed culmen, ± 1 mm) and tarsus length (± 0.1 mm) where possible (Fig. 5.1). For the analysis, we pooled the data gathered from these skins with the contemporary dataset.

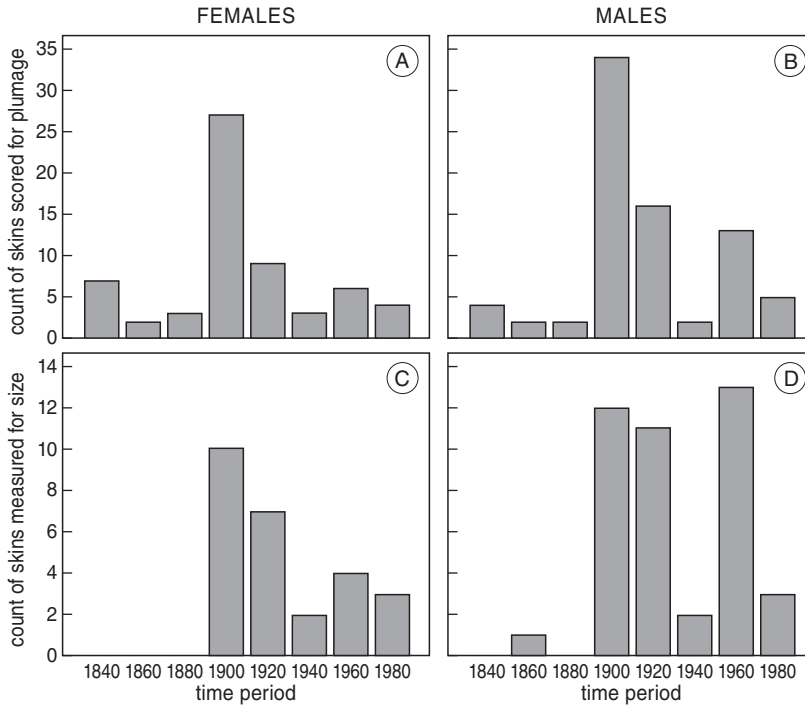


Figure 5.1: Numbers of male and female black-tailed godwit museum skins measured for plumage ornamentation (top) and size (bottom) per twenty-year period.

Table 5.1: Sample size and period of collected black-tailed godwit museum skins.

| | Female | | Male | |
|--|-----------|------------------|-----------|------------------|
| | N | period | N | period |
| Fries Natuurmuseum, Leeuwarden, The Netherlands | 3 | 1959–1997 | 2 | 1995–1997 |
| National Natural History Museum Naturalis, Leiden, The Netherlands | 30 | 1840–1997 | 34 | 1840–1981 |
| Zoological Museum Copenhagen, Denmark | 18 | 1901–1931 | 24 | 1901–1929 |
| Zoologisch Museum Amsterdam, The Netherlands | 10 | 1932–1986 | 19 | 1929–1982 |
| Total | 61 | 1840–1997 | 79 | 1840–1997 |

Plumage scores

We used four plumage scores that could be scored in both, the contemporary and the historical dataset. (1) *Bars* describes on a scale from one to five how far the black barring extends on a birds' breast and belly, (2) *Orange* describes the intensity of orange on its breast on a scale from one to five. (3) *White on head* is the percentage of white plumage in the head when seen in profile. (4) *Back* describes how much of the back of a bird is covered by breeding feathers in relation to retained winter feathers on a scale from one to five. We chose for these four scores because they have been shown to exhibit sexual dimorphism in black-tailed godwits, which suggests that these traits have been sexually selected for (Schroeder *et al.*, 2008a). In addition, we know that some of these traits may have a genetic basis (Schroeder *et al.*, 2008a).

All photos were scored blindly by JS, and as a control, by PML. We showed earlier that this method of plumage assessment in godwits has a high repeatability within and between different observers (for more details see Schroeder *et al.*, 2008a). Although a more precise quantitative method of plumage assessment may seem preferable, the spotted and sometimes irregular plumage of godwits makes it difficult to reliably assess color with a spectrophotometer. In addition, depending on how museum skins are mounted or not prevents taking pictures in a standardized position corresponding to living birds. If taken by a trained observer, the scores we use here are highly repeatable independent of the birds position, and thus enhance our confidence in a valid comparison of museum skins and living birds.

Data preparation

We collapsed bill length and tarsus length in a principal component analysis of body size (PCA). Wing length was not used because it is known to change its value post-mortem, and therefore the correlation between bill and tarsus and wing is supposed to differ between museum skins and living birds (Engelmoer *et al.*, 1983). The first principal component (PC1) explained 87% of the variation in tarsus and bill length, which load positively on PC1. In the following, this variable is used as an index of body size.

Variation in body mass can be the result of variation in size or variation in stores (van der Meer & Piersma, 1994) and may vary over the course of the season. To estimate size-corrected body mass (hereafter called 'condition'), we carried out a step-wise linear regression with body mass as dependent variable and the above mentioned size and date of season as predictor variable. Body mass was highly correlated with body size (linear regression: $F_{1,175} = 198.16$, $R^2 = 0.53$, $P < 0.001$), but not with date of season ($F_{2,174} = 0.01$, $R^2 = 0.001$, $P = 0.91$). We used the standardized residuals of this analysis as an index of condition.

We aggregated all plumage variables in a PCA due to strong multicollinearity between the scores (Schroeder *et al.*, 2008a). PC1 and PC2 explained 74% of the variation in plumage traits. Because we only found significant correlations with PC1, in the rest of the text we do not mention PC2. We refer to PC1 as plumage coloration, and high values stand for more orange and bars on the belly, and more breeding feathers on the back, and less white on the head (loadings: bars: 0.78, orange: 0.72, white head:

-0.57, back: 0.79). PC2 positively covaried with white in head (bars: 0.49, orange: -0.28, white head: 0.71, back: 0.30). To give more insight about changes in each separate trait for both sexes we present GLM-analyses of individual plumage scores for both sexes together and correlations separately per sex.

Statistics

Plumage scores consisted of non-continuous data, therefore, where necessary, appropriate transformations were identified and applied prior to statistical tests. For all GLMs, we tested for the equality of variances and no assumptions were violated. In analyses of the contemporary dataset, we used only one data point per individual. We performed GLMs with a factor for year to account for differences in annual means and always included interactions with sex to test whether effects of plumage differed between the sexes. We assigned males a 1 and females a 0. In analyses including size as a covariate, we used relative size for each sex separately (size-mean per sex) because we were not interested in the sexual size dimorphism. We centered wing length by subtracting the mean from each data point and dividing by one standard deviation. Heavier and larger female godwits lay larger eggs (this study) and we therefore added condition, size and the two-way interactions between condition, size and sex as additional explanatory variables to the analysis of egg volume. Laying date was tested as a covariate in all contemporary models, because reproductive components may change over the season, as well as plumage characteristics. We randomly deleted one sex of each nest where both birds were caught and used only the remaining birds in the analysis with egg volume as response variable to avoid pseudoreplication.

The historical data set has the potential problem of selective sampling, especially if curators have changed selection criteria for which individuals were collected (e.g. from actively collecting specimens to reliance on birds occasionally found dead). Another probable source of bias is the difference in sample size for different years and sexes (Fig. 5.1). To statistically correct for any such bias we used linear mixed-effect models, employing year nested by collection as random effects on the intercept. We did not correct for location as all museum skins from Dutch museums originated from The Netherlands, and all museum skins from Denmark were collected in Denmark. Breeding plumage may fade over the course of the season. Therefore, we modeled calendar date as a covariate in all models with historical plumage data. We ran this model for each of the three datasets: (1) the full dataset with both historical and contemporary data, (2) to account for possible outliers, a reduced historical dataset without the earlier years (1860-2007), (3) a dataset without the contemporary data and without the outliers (1860-1997). In addition, we calculated correlations for the univariate plumage variables per sex over time. We then tested with a similar linear mixed model whether size is related to plumage ornamentation in the historical dataset.

The covariates date of season and in the contemporary models year and laying date were not significant in any of our models, we however added them to the final models to correct for possible bias. Similarly, the random effect terms (year and collection)

were never statistically significant but we nonetheless left them in the models to account for probable bias.

We used R.2.7.1 statistical software for Mac OS X (R Development Core Team, 2008) for all statistical analysis. The `lme` function with the maximum likelihood procedure (`nlme` package) was employed for fitting linear mixed-effects models (Crawley, 2007). The most parsimonious model calculated with the ML (maximum likelihood) method was selected by Akaike's information criterion (Akaike, 1973; Burnham & Anderson, 2002). We report parameter estimates \pm SE for and t-tests for the significance of each parameter coefficient when all other fixed effects are present in the model, using the REML (restricted maximum likelihood) method (Pinheiro & Bates, 2000). The `lme` function uses an approximation of the denominator degrees of freedom according to the number of observations and the number of groups at each level of random effects which is, although only an approximation, good enough to compute reliable probability values for data sets with our sample sizes and models with relatively simple hierarchical structure (Pinheiro & Bates, 2000). We used the `stepAIC` functions (base package) to find the most parsimonious linear model stepwise selected by AIC. We report the parameter estimates \pm SE and additionally give F-statistics to allow judgment of the importance of factors. We used the transformed variables when calculating model statistics, however in graphics raw data was used to enhance understanding.

Results

Contemporary data - plumage ornaments as quality signals

Less colorful male black-tailed godwits were larger and in better condition than more ornamented males (Fig. 5.2A, C, Table 5.2). Females showed no correlation between coloration and size/condition (Figs 5B, D). The interactions `size*sex` and `condition*sex` remained in the most parsimonious model, indicating that the differences between the sexes are significant and the parameter estimates for both variables are similar (Table 5.2). When considering single traits, we found that less intensely orange-colored males were larger and in a better condition than more colorful males. Also, males with less breeding feathers on their backs are larger than more ornamented males (Table 5.3). The confidence intervals for the parameter estimates in these models were small. Paler males seem to be physically superior, because they are bigger and in better condition during incubation compared with more colorful males.

Paler godwit males with respect to PC1 were paired to partners that laid larger eggs than more ornamented males (Fig. 5.3A, Table 5.4). Larger females in a better condition laid bigger eggs; no effects of size and condition of males were found (Fig. 5.3 C-F, Table 5.4). The effect of male plumage on egg size is also supported in the analysis of single plumage traits: males with less barring were mated with females that laid larger eggs (Table 5.5). In the most parsimonious model for the univariate plumage scores, the interactions of bars, and orange with sex remained in the model (Table 5.5).

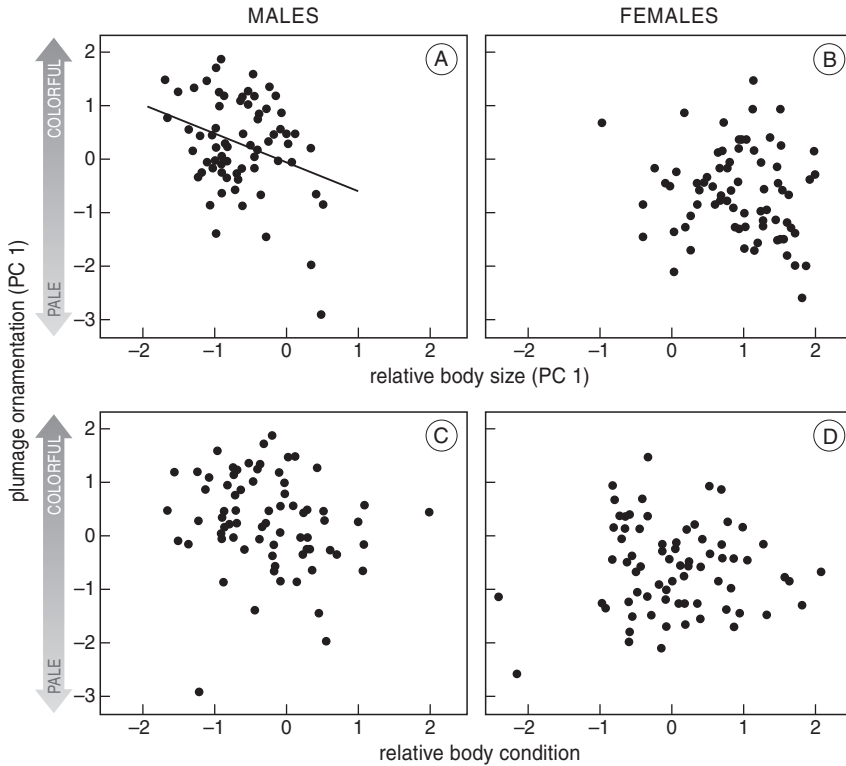


Figure 5.2: Relationship between plumage ornamentation (PC1) of male (A and C) and female (B and D) contemporary breeding black-tailed godwits size (PC1 combining bill and tarsus length) and condition (regression residuals of body mass on size). A: $F_{1,70} = 7.50$, $R^2 = 0.10$, $P = 0.008$; B: $F_{1,72} = 1.61$, $R^2 = 0.02$, $P = 0.21$; C: $F_{1,70} = 1.93$, $R^2 = 0.03$, $P = 0.17$; D: $F_{1,70} = 0.02$, $R^2 = 0.001$, $P = 0.89$.

Table 5.2: Results of the final model of model selection of GLMs with the first principal component (PC1) describing plumage ornamentation of contemporary breeding Black-tailed Godwits as dependent variables. A high principal component score describes a more ornamented bird. Sex, size, condition, and the interaction of condition and size with sex were modeled as explanatory variables. Displayed are parameter estimates ($\beta \pm \text{SE}$) of variables that were in the most parsimonious model, and F-statistics. Year and laying date were added as covariates to the most parsimonious model, but were not significant and are not displayed. $R^2 = 0.31$, $F_{7,136} = 8.73$, $P < 0.001$. The parameter estimate for males is given relative to females.

| y | β | $\beta \pm \text{SE}$ | F | P |
|------|---------------|-----------------------|-------|--------|
| PC 1 | Size | -0.13 ± 0.16 | 4.96 | 0.02 |
| | Sex | 0.84 ± 0.14 | 37.87 | <0.001 |
| | Condition | 0.01 ± 0.13 | 8.40 | 0.004 |
| | Size*sex | -0.48 ± 0.26 | 3.59 | 0.06 |
| | Condition*sex | -0.33 ± 0.19 | 3.11 | 0.08 |

Table 5.3: Results of the most parsimonious model of model selection of GLMs with the univariate plumage scores of contemporary breeding Black-tailed godwits as dependent variables. Sex, size, condition, and the interaction of condition and size with sex were modeled as covariates. Displayed are parameter estimates and F-statistics. Year and laying date were added as covariates to the most parsimonious model, but were not significant and are not displayed. Bars: $R^2 = 0.16$, $F_{5,136} = 5.13$, $P < 0.001$; orange: $R^2 = 0.29$, $F_{7,134} = 7.78$, $P < 0.001$; white head: $R^2 = 0.07$, $F_{3,138} = 3.38$, $P = 0.02$; back: $R^2 = 0.22$, $F_{6,135} = 6.33$, $P < 0.001$. The parameter estimate for males is given relative to females.

| | Bars | | | Orange | | |
|---------------|------------------|-------|--------|------------------|-------|--------|
| | $\beta \pm SE$ | F | P | $\beta \pm SE$ | F | P |
| Sex | 0.72 ± 0.19 | 16.34 | <0.001 | 0.36 ± 0.11 | 13.56 | <0.001 |
| Size | -0.29 ± 0.16 | 3.41 | 0.07 | 0.15 ± 0.14 | 2.26 | 0.13 |
| Conditon | -0.18 ± 0.13 | 4.80 | 0.03 | 0.09 ± 0.13 | 2.75 | 0.10 |
| Size*Sex | | | | -0.61 ± 0.22 | 7.47 | 0.007 |
| Size*Conditon | | | | -0.51 ± 0.17 | 5.47 | 0.02 |

| | White head | | | Back | | |
|---------------|------------------|------|-------|------------------|------|--------|
| | $\beta \pm SE$ | F | P | $\beta \pm SE$ | F | P |
| Sex | -0.96 ± 0.36 | 7.54 | 0.006 | 0.71 ± 0.15 | 24.6 | <0.001 |
| Size | | | | 0.05 ± 0.17 | 2.30 | 0.07 |
| Conditon | | | | -0.22 ± 0.11 | 4.07 | 0.05 |
| Size*Sex | | | | -0.63 ± 0.27 | 5.54 | 0.02 |
| Size*Conditon | | | | | | |

Historical data - change plumage coloration and body size since 1840

We find that male black-tailed godwits changed to a less colorful plumage between 1840 and 2007, in contrast to females that did not show a change (Table 5.6, Fig. 5.4). Year squared and the interaction between year squared and sex were always removed from the model with the lowest AIC. This model contained the interaction between sex and year, confirming that plumage ornamentation changed only in males, but not in females. However, in more restricted datasets (2 and 3), only the main effects year and sex remained in the most parsimonious model, without a significant difference in slope between the sexes (interaction sex*year when in the model: (2): $t = -0.73$; $P = 0.47$; (3): $t = -0.04$; $P = 0.97$; Table 5.6), suggesting that plumage became paler in both sexes. This may be because from 1950 to 2003 we had only 11 female skins samples (males $N = 20$), which were all relatively pale in comparison to the average female plumage (mean female $PC1 \pm SD$: -0.54 ± 0.89 , mean female $PC1$ 1950–1997: -0.75 ± 0.84). To account for this, we ran a model in a fourth dataset, in which we excluded data from after 1950 (Table 5.6). In this model, the interaction between year

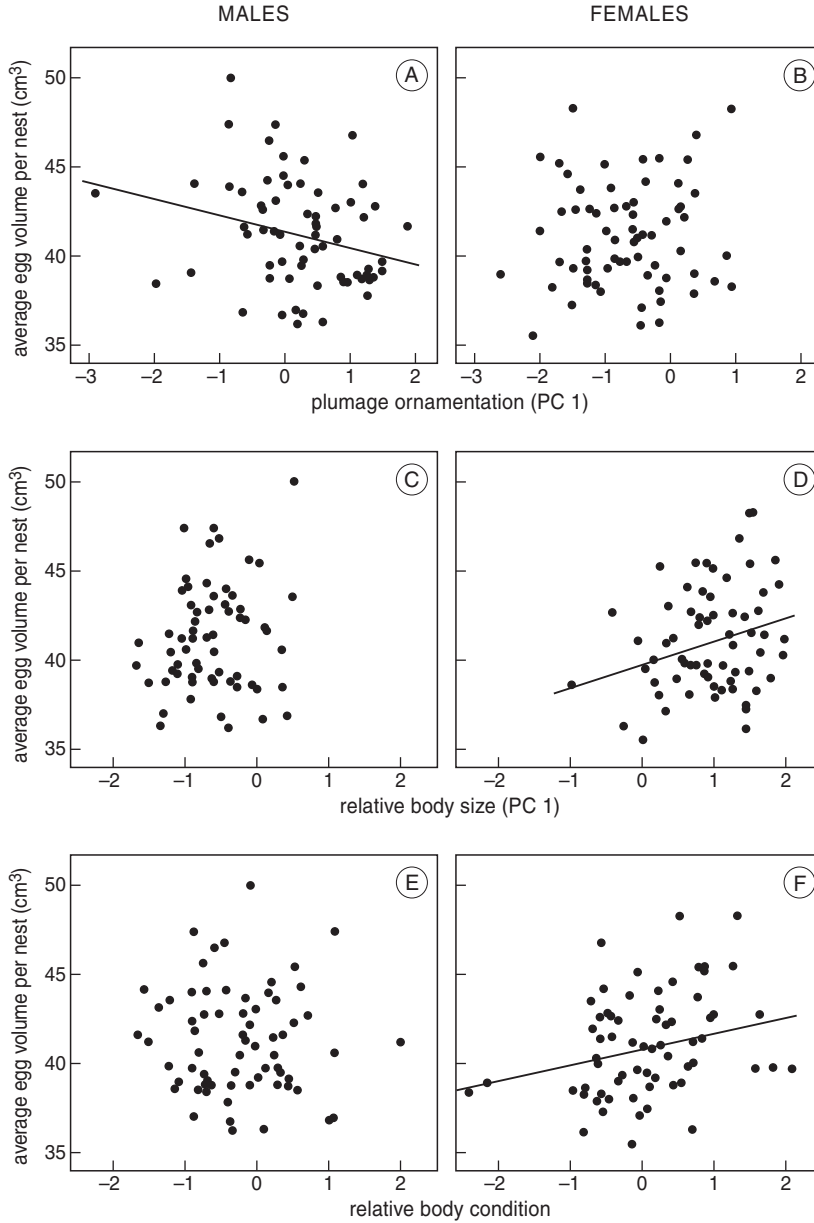


Figure 5.3: Correlation between plumage ornamentation (A and B), size (C and D) and size corrected body mass (E and F) and average egg volume per nest of contemporary male and female black-tailed godwits breeding in The Netherlands. A: $F_{1,67} = 4.63$, $R^2 = 0.06$, $P = 0.04$; B: $F_{1,67} = 0.24$, $R^2 = 0.01$, $p = 0.63$; C: $F_{1,67} = 0.76$, $R^2 = 0.01$, $P = 0.39$; D: $F_{1,67} = 5.15$, $R^2 = 0.07$, $P = 0.03$; E: $F_{1,67} = 0.19$, $R^2 = 0.01$, $P = 0.66$; F: $F_{1,65} = 3.61$, $R^2 = 0.05$, $P = 0.06$.

Table 5.4: Result of the final model of model selection of GLMs with average egg volume of Dutch Black-tailed godwit nest as dependent variable. PC1, PC2, size, condition, sex and the two-way interactions between PC1, PC2, size and condition with sex were modeled as explanatory variables. Year (as a factor) and laying date were added to the most parsimonious model, but were not significant and are not displayed. $N = 57$ females, 60 males. $R^2 = 0.14$, $F_{11,103} = 2.74$, $P = 0.004$. Sex: female = 0, male = 1.

| Variable | $\beta \pm SE$ | F | P |
|------------------------|------------------|------|------|
| PC1 | 0.49 ± 0.53 | 0.86 | 0.35 |
| size | 1.88 ± 0.65 | 4.89 | 0.03 |
| condition | 1.77 ± 0.58 | 0.98 | 0.32 |
| sex | 0.76 ± 0.71 | 0.22 | 0.64 |
| PC1 \times sex | -1.65 ± 0.73 | 5.12 | 0.02 |
| size \times sex | -2.96 ± 1.11 | 4.76 | 0.03 |
| condition \times sex | -2.53 ± 0.81 | 4.95 | 0.03 |

Table 5.5: Result of the final model of model selection of GLMs with average egg volume per Dutch Black-tailed godwit nest as dependent variable. All plumage variables, size, condition, sex and the two-way interactions between the plumage scores, size and condition with sex were modeled as covariates. Year (as a factor) and laying date were added to the most parsimonious model, but were not significant and are not displayed. $N = 57$ females, 60 males. $R^2 = 0.14$, $F_{13,101} = 2.34$, $P = 0.006$. The parameter estimate for males is given relative to females.

| Variable | $\beta \pm SE$ | F | P |
|------------------------|------------------|------|------|
| bars | 0.45 ± 0.42 | 0.49 | 0.48 |
| orange | 0.17 ± 0.64 | 0.59 | 0.44 |
| condition | 1.83 ± 0.61 | 0.98 | 0.32 |
| size | 1.99 ± 0.68 | 4.89 | 0.03 |
| sex | 8.54 ± 3.13 | 0.22 | 0.64 |
| bars \times sex | -1.15 ± 0.55 | 4.40 | 0.04 |
| orange \times sex | -0.96 ± 0.82 | 3.03 | 0.08 |
| size \times sex | -2.90 ± 1.11 | 4.52 | 0.04 |
| condition \times sex | -2.58 ± 0.84 | 4.57 | 0.03 |

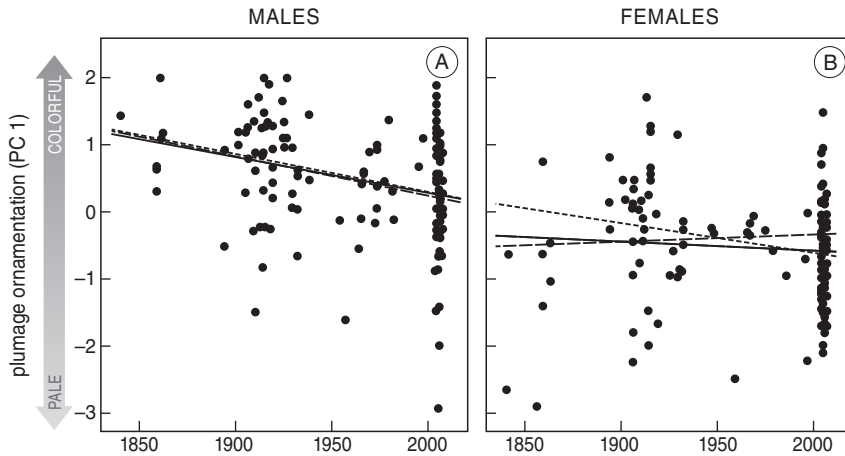


Figure 5.4: The historical development of black-tailed godwit plumage ornamentation in museum skins and contemporary breeding birds. Year is the year or capture for the contemporary data (2004–2007) or the year of death in the historical data. Straight lines are regression lines for the full dataset (1840–2007), dashed lines refer to data from 1860–2007, dotted lines to data from 1860–1997. Males, 1840–2007: $F_{1,149} = 14.22$, $R^2 = 0.09$, $P < 0.001$; 1860–2007: $F_{1,145} = 13.95$, $R^2 = 0.09$, $P < 0.001$; 1860–1997: $F_{1,77} = 5.88$, $R^2 = 0.07$, $P = 0.02$. Females, 1840–2007: $F_{1,133} = 0.85$, $R^2 = 0.01$, $P = 0.36$; 1860–2007: $F_{1,126} = 5.97$, $R^2 = 0.05$, $P = 0.02$; 1860–1997: $F_{1,59} = 0.11$, $R^2 = 0.01$, $P = 0.74$.

and sex remained in the most parsimonious model. The analyses for all four datasets imply that whether or not the interaction between sex and year remains in the model hinges on contradictory trends in females (Fig. 5.4B), while the change in frequency of colorful males is pronounced in whatever data selection (Fig. 5.4A, Table 5.6).

For single plumage traits we found that bars score, orange score and back score decreased over time in males, and the amount of white in head plumage increased (Fig. 5.5). Bars score and back score in males decreased on average by about 20% over 164 years. White in head increased over time in females, too (Fig. 5.5). Overall, we find strong evidence for a change in frequency of colorful and ornamented males towards paler males over time.

We also examined changes in size over time. In all datasets, we found that both males and females increased in size over time to a similar extent (Table 5.6). However, when excluding the contemporary birds, the parameter estimate for year had a larger confidence interval and the change in size was a mere trend, suggesting that the difference is mainly between museum specimens and living birds (Table 5.6), which most likely is a storage effect. Year squared and the interaction between year squared and sex never remained in the most parsimonious model. A similar analysis on the full dataset revealed that wing length also increased over the years in both sexes, and the interaction was removed from the most parsimonious model (Full dataset, $\beta \pm \text{SE}$: year:

Table 5.6: Results of the most parsimonious models of mixed linear model selection where ornamentation and size of Black-tailed godwit museum skins and contemporary breeding birds were modeled as dependents. For each model we modeled year nested in museum collection as random effects. Year, sex and the interaction of year \times sex were modeled as explanatory variables. Date of capture was modeled as covariate in the models on plumage ornamentation. It was never significant. The parameter estimate for males is given relative to females.

| | Body size | | | Plumage ornamentation (PC 1) | | |
|-------------------|---------------------------------------|--------|--------|--------------------------------------|-------|--------|
| | $\beta \pm \text{SE}$ | t | P | $\beta \pm \text{SE}$ | t | P |
| | full dataset (1840–2007), N = 216 | | | full dataset (1840–2007), N = 286 | | |
| year | 0.003 \pm 0.001 | 2.82 | 0.008 | –0.001 \pm 0.002 | –0.56 | 0.58 |
| sex | –1.58 \pm 0.08 | –20.61 | <0.001 | 8.42 \pm 3.40 | 2.11 | 0.04 |
| sex \times year | - | - | - | –0.004 \pm 0.002 | –1.96 | 0.05 |
| | without outliers (1860–2007), N = 215 | | | without outliers (1860–2007), N=275 | | |
| year | 0.003 \pm 0.001 | 2.64 | 0.01 | –0.006 \pm 0.002 | –3.33 | 0.002 |
| sex | –1.58 \pm 0.08 | –20.53 | <0.001 | 0.95 \pm 0.10 | 9.64 | <0.001 |
| sex \times year | - | - | - | - | - | - |
| | historical only (1860–1997), N = 69 | | | historical only (1860–1997), N = 129 | | |
| year | –0.005 \pm 0.002 | –1.79 | 0.08 | –0.006 \pm 0.003 | –2.37 | 0.02 |
| sex | –1.66 \pm 0.13 | –13.02 | <0.001 | 1.03 \pm 0.14 | 7.36 | <0.001 |
| sex \times year | - | - | - | - | - | - |
| | (1840–1950), N = 109 | | | | | |
| year | 0.01 \pm 0.005 | 2.45 | 0.02 | | | |
| sex | 29.52 \pm 13.07 | 2.26 | 0.03 | | | |
| sex \times year | –0.01 \pm 0.007 | –2.17 | 0.03 | | | |

0.004 \pm 0.002, $t = 2.41$, $P = 0.02$; sex: -1.35 ± 0.09 , $t = -15.37$, $P < 0.001$, $N = 216$ observations), and in all datasets without the contemporary birds, year did not remain in the most parsimonious model. Thus, museum skins were smaller than live birds, whereas we cannot conclude that the godwits have changed in size.

In contemporary birds, we found a correlation between size and plumage coloration, but for the historical material, we did not find that paler birds were bigger (only sex remained in the most parsimonious model, $\beta \pm \text{SE}$: sex: 1.10 ± 0.15 , $t = 7.59$, $P < 0.001$, $N = 140$ observations). The three-way interaction (year*sex*size) was rejected, as were all two-way interaction of the three variables.

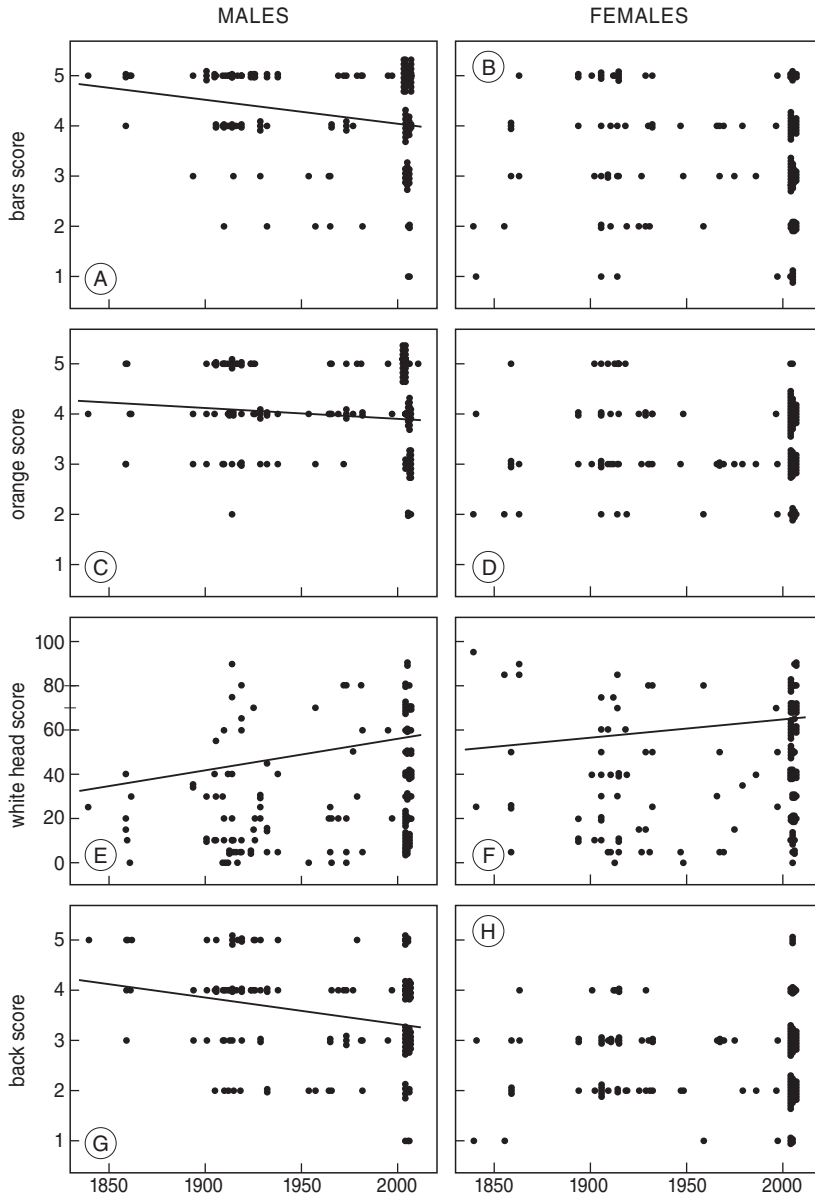


Figure 5.5: The historical development of univariate plumage scores of black-tailed godwit museum skins and contemporary breeding birds. Year is the year of capture for the contemporary data (2004–2007) or the year of death. Data points with the same value were jittered. $N = 135$ females, 151 males. Spearman correlations: A: $\rho = -0.22$, $P = 0.008$; B: $\rho = -0.13$, $P = 0.15$; C: $\rho = -0.27$, $P < 0.001$; D: $\rho = -0.05$, $P = 0.54$; E: $\rho = 0.28$, $P < 0.001$; F: $\rho = 0.18$, $p = 0.03$; G: $\rho = -0.24$, $P = 0.002$; H: $\rho = -0.01$, $P = 0.88$.

Discussion

WE SHOWED THAT black-tailed godwit males with a paler breeding plumage were larger, in better condition and paired with females that laid larger eggs compared to more colorful males. We found no such effects in females. If pale males indeed consistently perform better than colorful males, we expect the population to change into a paler plumage over the years. This was supported by the analysis of plumage coloration using museum specimens: we found weakening of male coloration towards a more female-like plumage since 1840 (Figs 5.4 and 5.5). One explanation for this may be negative directional selection on male coloration and relaxation of selection pressures on sexual dimorphism in plumage. We will discuss this and other possible explanations in the next paragraphs.

Pale males could be pale because (1) they molt fewer feathers from winter to breeding plumage (see, e.g. Jukema & Piersma, 2000; Battley *et al.*, 2006) or (2) the feathers are replaced by alternate, but less pigmented feathers. Studies on a related shorebird in captivity, the red knot *Calidris canutus*, demonstrate that during a pre-alternate molt birds rarely, if ever, grow a combination of pigmented and unpigmented feathers (T. Piersma & M. Brugge unpubl. data). In addition, in an unsystematic study, back feathers in active molt of captive black-tailed godwits during pre-alternate molt were always ornamented (J. Schroeder & F. Santiago-Quesada unpublished data). However, in the absence of detailed studies (e.g. Reneerkens & Korsten, 2004), we presently cannot distinguish between an increasing numbers of retained basic feathers and decrease of feather pigmentation without a change in molt intensity.

We report an increase in frequency of less colorful godwit museum skins over a period of 164 years, most prominently in male godwits. This is largely based on museum skins and relies on the assumption that the observed change in plumage is independent of storage time and that birds were collected as random samples of the population. If the first assumption would be false, we expect older skins to have faded more, which is against the expected trend. The second assumption of random sampling of the population is impossible to test: if the most ornamented individuals in the past were selectively collected, whereas in recent years museums rely on birds that are found occasionally dead, this would lead to the observed trend. We do not find an effect of collection, which, since skins are divided among themselves roughly by time (C. Roselaar, personal comment; Table 5.1), would be expected if collection practice over time were a prominent factor.

Paler contemporary males were also found to be bigger in body dimensions, giving the possibility that selection may act also on the correlated size traits. For this we examined whether a change in size could be detected using the museum specimens, but these analyses were ambiguous. Wing length decreased over time in both sexes as expected from earlier work on shrinkage of museum skins (Engelmoer *et al.*, 1983), but we found no clear trend in the compound measure of bill and tarsus length over time. We thus cannot exclude that the change of size over time is an artifact (Table 5.6), and although the correlation between coloration and size in males post

1960 supports it we cannot demonstrate that male godwits are becoming larger over time.

It has been suggested that breeding plumage of godwits may fade with age (N. M. Groen, pers. comm.). Black-tailed godwits have experienced a strong population decline during the last fifty years (Beintema *et al.*, 1995), and as this decline is due to lowered reproductive output and not to lowered adult survival (Gill *et al.*, 2007; Roodbergen *et al.*, 2008), the average age of the population may well have increased. A pattern of older, paler males mated with females who lay larger eggs may arise if godwits mate assortatively by age (Black & Owen, 1995), and older and more experienced females invest more into reproduction (Sæther, 1990; Fasola *et al.*, 2001). However, between-year repeatability of plumage scores of individuals is relatively high (Schroeder *et al.*, 2008a). There is no systematic study that shows an effect of age on plumage, and therefore we cannot reject that ageing of the population may have contributed to the change in plumage coloration.

Our results suggest that paler and larger males are paired with, or chosen by, females that are able and willing to invest more into reproduction and are likely to be of 'high quality' themselves. Our data of less ornamented males being of higher quality goes against the traditional view of sexual selection as a unidirectional process towards ever more elaborate male plumage traits (Andersson, 1994). Two studies have reported comparable effects of lesser-ornamented male birds gaining reproductive benefits in a contemporary dataset. To avoid hybridization, pied flycatcher *Ficedula hypoleuca* females prefer duller brown males in areas where they breed in sympatry with the collared flycatcher *F. albicollis*, whereas in areas of allopatry, more ornate males were preferred (Sætre *et al.*, 1997). This effect was geographically restricted and unlikely to change the mean population trait over the entire range. In an island population of house sparrows *Passer domesticus*, females preferred to mate with males with a smaller black badge, as opposed to several mainland populations (Griffith *et al.*, 1999; but see Nakagawa *et al.*, 2007). On the island, male parental care was of greater importance, and females were interpreted to maximize reproductive success by choosing the less ornate males that invested most in paternal care (Griffith *et al.*, 1999). The proximate mechanism may be that testosterone levels are positively associated with large bibs, male-male competition but also bad parental care (Hegener & Wingfield, 1987).

Against a background of numerous studies showing fitness benefits of more elaborate plumage coloration, we explore further explanations for the apparent reduction in sexual plumage dimorphism in this subspecies of black-tailed godwits. Changes in selection pressures maintaining sexual dimorphism can be evoked by environmental changes, and both breeding and non-breeding habitat of black-tailed godwits has changed strongly during the last two centuries (Beintema *et al.*, 1995), attributable to increased industrialization of agricultural practices. Black-tailed godwits had increasingly to rely on agricultural — man-made — habitats. Until the start of the 20th century, the species bred mostly in fen and bog areas, which were changed into agricultural grasslands concomitant with the reported change in plumage (Beintema *et al.*, 1995; Fig. 5.4). The godwits first profited from this change, and their numbers

increased because densities on extensive agricultural land were higher than on the former, more natural habitats, likely due to the higher food availability in man-made and nitrogen-enriched grasslands (Beintema *et al.*, 1995). Godwit males display and defend their territory by aerial displays, which are assumed more effective when males are smaller, more agile and more ornamented (Jönsson & Alerstam, 1990). The availability of better quality territories may have led to a decrease in male-male competition during the first half of the 20th century. Therefore, visual signals based on plumage and aerial displays may have become less important.

The black-tailed godwit also experienced a drastic change in non-breeding habitat on the spring staging areas where they molt into breeding plumage, and hence changes at this phase could have had a direct effect on plumage. From 1920 onwards, many natural wetlands on the Iberian Peninsula have dried out and at the same time, formerly dry land has been remodeled into rice cultivations (Lains & Sousa, 1998; Sánchez-Guzmán *et al.*, 2007). Historically, godwits staged in natural freshwater wetlands where they probably ate a large proportion of animal food. In contrast, at present during spring stopover they forage mainly on spilled, and sometimes burned, rice grains in large rice fields (Sánchez-Guzmán *et al.*, 2007). Maybe the change from a carnivorous to a granivorous diet made molt more costly (Lindström *et al.*, 1993). If the acquisition of a full, bright alternate plumage on a rice diet is indeed costly, males who trade plumage ornamentation for body condition may gain an advantage with respect to timing of migration, and prevent the concurrent costs of late reproduction (Roodbergen & Klok, 2008). Irrespective of quality, quantity of food available to godwits at agricultural staging sites may be higher than in natural wetlands, and it may be that also here interspecific competition for food decreased with the higher abundance of rice fields.

A potential proximate explanation is the positive correlation between melanin plumage ornamentation and testosterone levels between species (Bókony *et al.*, 2008). If this correlation also exists intraspecifically, a decrease in general and male-male competition may be linked to a decrease in overall testosterone levels (Jawor, 2007) and could be a proximate mechanism for a phenotypic reduction of male melanin ornamentation (Ducrest *et al.*, 2008; Rubenstein & Hauber, 2008). This phenotypic change could be accompanied by a genetic change, especially if size and plumage traits are genetically correlated, which may be expected in species with a size and plumage sexual dimorphism. This could over time lead to a loss of sexual plumage dimorphism if changes in selection pressures for less ornamented males lead ultimately to a change in the slope of the reaction norm of melanin coloration on competition intensity. The strong decrease in population size in the last decades due to lowered reproductive success may have enhanced the selection pressure and facilitated microevolutionary processes.

This correlational study shows an increase of paler male black-tailed godwits, which may be due to an ageing population, but more likely represents a change of sexual plumage dimorphism over 167 years. That egg volumes is greater in pairs with paler males, and that females do not seem to pale over time provides supporting, yet

circumstantial, evidence for this possibility. Changes in selection pressures that affect male godwit ornamentation may be due to large-scale anthropogenic land use changes throughout the range of black-tailed godwits.

Acknowledgements

We want to thank It Fryske Gea, Fûgelwacht Warkum, Niko Groen, Petra de Goeij, Valentijn van den Brink, Rosemarie Kentie and Freek Mandema for invaluable help in the field. Our thanks go to Jon Fjeldså (Zoological Museum Copenhagen), Hein van Grouw (Naturalis, Leiden), Peter Komen (Fries Natuurmuseum, Leeuwarden) and Kees Roselaar (Zoologisch Museum Amsterdam) for allowing us access to their natural history collections. We thank Jenny Gill, Will Cresswell, Martin Hinsch and one anonymous reviewer for constructive comments on earlier versions of the manuscript and Dick Visser for preparation of the figures.

Six

Intrasexual competition predicts a positive relation between plumage ornamentation and female reproductive investment, but not with timing of breeding in the Icelandic black-tailed godwit

Julia Schroeder, Maja Roodbergen, Jordi van Gestel,
Valentijn van den Brink, Niko M. Groen & Gerrit J. Gerritsen

Abstract

Colorful breeding plumage ornamentation is widespread among birds. Males are often more ornamented than females, and such sexual dimorphism may have arisen through sexual selection, driven by female choice (intersexual) and male-male (intrasexual) competition. However, in the nominate species of the sexually dimorphic black-tailed godwit (*Limosa l. limosa*), paler males are mated with females that invest more into reproduction, possibly due to reduced male-male competition for high quality (concerning adult food availability pre-egg laying) breeding sites. If indeed male-male competition for nesting sites drives sexual selection of plumage ornamentation in godwits, we expect it to be positively correlated with female reproductive investment in populations experiencing strong male-male competition. We test this in the Icelandic subspecies (*L. l. islandica*), where a population increase has led to enhanced competition for nesting sites among males. This should have amplified selection for more ornamented males in the Icelandic subspecies. We test whether more ornamented Icelandic male godwits are mated with females that lay earlier and larger eggs. We assessed the extent and number of breeding feathers and orange breast coloration in males on the basis of photos taken of birds captured on the nest in 1993 and videos of birds returning to their nests in 2008. We found no relationship between male breeding plumage ornamentation and timing of egg laying. However, more ornamented males were mated with females that laid larger eggs in both years, opposite to the pattern found in the nominate species. This suggests that male plumage ornamentation may indeed be a sexually selected signal in godwits, and that intraspecific competition may be important.

Introduction

MANY BIRD SPECIES SHOW CONSPICUOUS plumage ornamentation and sexual plumage dimorphism (Hill & McGraw, 2006). The theory of sexual selection predicts that sexual dimorphism can be due to either males signalling their ability to compete for mates and territories (male-male, or intrasexual competition) or their reproductive qualities to prospective mates (female choice, or intersexual competition) (Andersson, 1994). To be reliable, such signals should be costly (Zahavi, 1975; Pomiankowski, 1988). Indeed, more exaggerated, colorful and conspicuous plumage ornamentation in males is often positively correlated with male condition, male-male competitive abilities, female choice and reproductive investment, reproductive success and male viability (for reviews see Andersson, 1994; Hill, 2002; Hill & McGraw, 2006).

In migratory birds, early arriving males are better able to secure territories of high quality (Kokko, 1999). Further, early arrival on the breeding grounds in godwits and other long-distance migrants is often related to an earlier onset of breeding, and in turn to a higher reproductive output (Drent *et al.*, 2003; Bêty *et al.*, 2003). To arrive early, birds must be able to migrate faster or leave the staging grounds earlier (Drent *et al.*, 2003). Such migratory quality of male birds can be signaled by plumage ornamentation (Piersma & Jukema, 1992; Piersma *et al.*, 2001; but see Drent *et al.*, 2003).

Both the European mainland subspecies (*Limosa limosa limosa*), and the Icelandic subspecies of the black-tailed godwit (*L. l. islandica*) are sexually dimorphic for breeding plumage and size (Groen & Yurlov, 1999; Gunnarsson *et al.*, 2006a; Schroeder *et al.*, 2008). This migratory shorebird winters in Western Europe and breeds almost exclusively in Iceland (Gill *et al.*, 2007). Godwits secure their future nesting site soon after arrival on the breeding grounds, and stay there for the whole pre-breeding period (Van den Brink *et al.*, 2008). Males display to females and compete with other males by aerial and ground displays and fights (Cramp & Simmons, 1983), which are assumed to be more effective when males are more ornamented (Jönsson & Alerstam, 1990).

Surprisingly black-tailed godwit males of the nominate race with a less colorful plumage were mated with females who laid larger eggs, and currently, the sexual plumage dimorphism is less pronounced than it was 150 years ago, males nowadays looking more alike females (Schroeder *et al.*, 2009). This contradictory pattern has been attributed to large-scale land-use changes on the breeding grounds in The Netherlands, where a large part of the *limosa* population breeds. The higher food availability in nitrogen-enriched grasslands (Beintema *et al.*, 1995) likely led to a decrease in male-male competition for nesting sites, which was suggested to have led to a relaxation of selection pressures via dynamic feedback interplay between behavior, hormone levels and melanin coloration (Ducrest *et al.*, 2008; Rubenstein & Hauber, 2008; Safran *et al.*, 2008). In turn, male plumage ornamentation may have become less important in the *limosa* subspecies, benefiting males who did not invest in a costly colorful breeding plumage (Schroeder *et al.*, 2009).

Contrastingly, Icelandic godwits experienced a remarkable population increase, over the last century, and more low quality habitat was occupied, both on the

breeding and wintering grounds (Gunnarsson *et al.*, 2005a; -b). On the population level, *islandica* males who arrive earliest on Iceland after spring migration secure territories in high quality breeding habitat and subsequently benefit from high reproductive success (Gunnarsson *et al.*, 2005a; -b). This leads to the conclusion that, on an individual level, male-male competition for nesting sites should be relatively strong, at least on high quality breeding grounds in Icelandic godwits. And if in the *islandica* subspecies, too, male plumage ornamentation plays a role in intrasexual competition, we expect that in *islandica* godwits, male ornamentation should be positively related to timing of breeding and correlates of reproductive success. In this study, we examined the correlations between sexually dimorphic breeding plumage traits of male Icelandic black-tailed Godwit and measures of reproductive success (egg volume) and timing of breeding.

Methods

Fieldwork

We gathered data on breeding godwits during two field trips to Iceland in 1993 and 2008 in high quality breeding grounds. In 1993, GJG and NMG collected data on 34 godwit nests on the lowland grassland area between the towns of Selfoss (S, 63°56'N; 21°00'W) and Eyrarbakki (E, 63°52'N; 21°11'W) in southern Iceland (see also Gerritsen & Groen, 1995). That year, 20 nests were located on the derelict air field in Kaldaðarnes (K, 63°55'N; 21°10'W), where Black-tailed godwits breed in a high density. This area and the surrounding estuarial grasslands belong to some of the oldest traditionally used nesting sites of the Icelandic black-tailed godwit (T.G. Gunnarsson, pers. comm.). In the Skogar reserve in north-Iceland (G, 65°45'N; 20°26'W), 14 additional nests were found. This area is a silted, fertile floodplain, and habitat quality is high. In total, that year 15 male and 22 female Icelandic godwits were caught on their nest with claptraps. Photographs with one wing stretched out were taken of each bird caught. At three nests both the male and the female were caught. On 34 nests, egg length and width was measured to the nearest mm and incubation status was determined by measuring the angle of a floating egg in water following van Paassen *et al.*, (1984).

In 2008, JS, MR, VvdB and JvG collected data of 17 godwit nests during the breeding season in the area of the derelict air field of Kaldaðarnes (see above). Eggs were treated similarly as in 1993, but adult birds were not caught. Instead, we made use of small, battery-powered digital camcorders that were placed in close proximity (1.5m) of the nest. These camcorders (Technaxx C-2000 and Technaxx C-8000) recorded the nest site and birds returning to their nests for 45 -125 minutes (for a detailed method description see Van den Brink *et al.*, 2008). We continued to place camcorders until we had video footage of both parents, and on average each nest was videotaped four times. This method allowed us to get data on both birds of 14 nests.

Plumage assessment

Experienced observers (JS and JvG respectively) scored three features of the plumage that have been shown to be sexually dimorphic in black-tailed godwits (Schroeder *et al.*, 2008): (1) orange intensity; the intensity of orange color on breast and neck, (2) back score; the proportion of pigmented breeding feathers on the back and wings of the bird in relation to the total amount, and (3) breeding feathers; the absolute number of breeding feathers on the back of the bird (for more detailed description, see Schroeder *et al.*, 2008). Photos and videotapes were scored (by JvG and JS) without previous knowledge of the reproductive parameters. Within and between observer-repeatability was high ($>0.9 \pm 0.1$ SE, for all variables calculated following Lessells & Boag, 1987). Although a more quantitative method of plumage scoring might seem preferable, this method allows us to compare data from different source media since the scores taken from photos and videos are repeatable within individual godwits of the nominate species (own unpublished data). Further, spectrophotometer measurements are not suitable for strongly heterogenic plumage coloration that contain small elements (dots) of different color in most traits, and are therefore not suitable for godwits (see Schroeder *et al.*, 2008). Sex was assigned based on morphological characteristics (Gunnarsson *et al.*, 2006a).

Data preparation and statistical methods

Egg volume was calculated by the formula $\text{length} \times \text{width}^2 \times 0.52$, (Romanoff & Romanoff, 1949). Egg volume is more variable than clutch size, because godwits nearly always lay a clutch of 4 eggs (Schroeder *et al.*, submitted). In this study, $>90\%$ of all nests contained four eggs. Larger godwit eggs hatch heavier chicks that have a higher chance of survival (own data, Schekkerman *et al.*, 2008). We had no data on individual fledging success, which cannot be reliably determined without the use of radio transmitters on chicks and adults (Roodbergen & Klok, 2008, Schekkerman *et al.*, 2008). For our purposes, we use egg volume as a correlate of reproductive success. Estimated laying date was calculated by subtracting the incubation (5 days) and laying period (23 days) from the hatching date (Beintema *et al.*, 1995). Egg volume can be affected by the time of season and we tested for the relationship between laying date and egg volume with a linear model where we modelled year and location as fixed effects on the intercept.

We calculated z-scores of all plumage scores per year by subtracting the population mean from an individual raw score to prevent bias due to two different media (Quinn & Keough, 2002). Due to strong multicollinearity, we collapsed all plumage traits in a principal component analysis. The first principal component explained 82% of the variation in plumage scores (eigenvalue = 2.45), and all scores load positively on this component (loadings: orange: 0.81, back: 0.95, breeding feathers: 0.95). Birds that score high on this first principal component (PC1) have a brighter orange plumage and their backs are covered to a larger extent and number in ornamented breeding. We assessed normality of PC1 visually (Gelman & Hill, 2006). In the following, we use this PC1 as an indication of plumage ornamentation in black-tailed godwits.

We tested for assortative mating on raw plumage traits between females and males incubating the same nest with Spearman's rank correlation test, and on PC1 with Pearson correlation (both two-tailed). We were interested in the relationship between male plumage ornamentation, timing of breeding and female reproductive investment. Since the relationship between reproductive success and plumage may depend on local conditions, like nest density or strength of male-male competition, we employed linear mixed models, where location (breeding area (S, E, K, G nested in north/south) were modeled as random effects on the intercept, year as fixed effect, and PC1 as covariate and the interaction between the latter two. The random terms were never significant, but we added them to the most parsimonious model to correct for any potential bias. Different plumage characteristics can signal different information (Doucet & Montgomerie, 2003). We therefore used univariate analyses to describe the relationship between male plumage and laying date and egg volume in detail. The plumage scores were intercorrelated, with birds that are strongly ornamented in one trait being also more colorful with respect to the other traits (Schroeder *et al.*, 2008). Therefore, if one would find a significant relationship with any given plumage score, it would be highly likely that a similar relationship is also present with the other scores.

We do not apply Bonferroni tests since those correct Type I errors (false positive) in multiple, independent tests. In the type of data we present here, however, it is more likely to get a significant correlation if another one is significant. To prevent this type of problem we use the first principal component in the first place and we use both univariate analysis and linear models to supplement each other. Statistics were calculated with R.2.7.1 statistical software for Mac OS X (R Development Core Team 2008). The `lme()` function with the maximum likelihood procedure (nlme package) was employed for fitting linear mixed-effects models and selected the most parsimonious model by Akaike's information criterion (Akaike, 1973; Burnham & Anderson, 2002), using the maximum likelihood method (ML) (Pinheiro & Bates, 2000). We report parameter estimates \pm SE for the most parsimonious model, now estimated using restricted maximum likelihood method (REML), and report the significance values of each parameter coefficient when all other parameters are present in the model (Pinheiro & Bates, 2000). We set $\beta = 0.05$. Sample sizes differ due to missing values.

Results

PLUMAGE ORNAMENTATION described by PC1 did not differ between birds from 1993 and 2008 (males: $t = -0.83$, $P = 0.41$, $N_{1993} = 13$, $N_{2008} = 8$; females: $t = 0.58$, $P = 0.57$, $N_{1993} = 18$, $N_{2008} = 10$). Males were more colorful than females with respect to PC1 (Figure 6.1; mean \pm SE: females: -0.44 ± 0.20 , males: 0.58 ± 0.11 , $t = -4.42$, $P < 0.001$, $N = 28$ females and $N = 21$ males). Males had more orange, a larger extent of breeding plumage and more breeding feathers on their backs (Wilcoxon-test: $W_{\text{orange}} = -250$, $N_{\text{females}} = 35$, $N_{\text{males}} = 29$, $P < 0.001$; $W_{\text{back}} = 295.5$, $N_{\text{females}} = 35$, $N_{\text{males}} = 29$, $P = 0.003$; $W_{\text{feathers}} = 154.5$, $N_{\text{females}} = 28$, $N_{\text{males}} = 22$, $P = 0.004$).

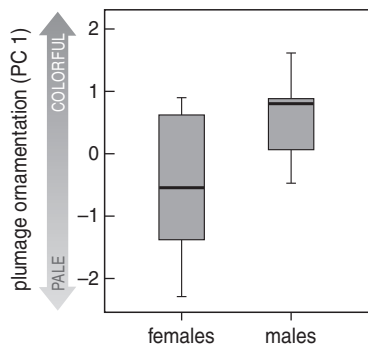


Figure 6.1: Plumage ornamentation (PC 1) of male and female Icelandic black-tailed godwits during breeding season. A high PC1 indicates a more ornamented breeding plumage. Whiskers depict the interquartile range of values (there where no outliers), the extent of the Boxes marks the lower and upper quartile and the line inside the Box gives the median value.

Egg volume was smaller in 2008 (2008: $40.25 \pm 0.58 \text{ cm}^3$, $N = 17$; 1993: $42.07 \pm 0.51 \text{ cm}^3$, $N = 33$, $t = 2.35$, $P = 0.02$). Laying date did not differ between the two years (28 May ± 1 day, $t = -0.55$, $P = 0.59$, $N = 51$). There was no change of average egg volume per nest over the course of the season (Figure 6.2, parameter estimate $\pm \text{SE}$: $\beta = 0.08 \pm 0.13$, $F_{1,31} = 0.36$; $P = 0.55$).

We found no assortative mating by PC1 and also not by raw plumage scores (Table 6.1). Male plumage ornamentation was not statistically significantly related to laying date (Figure 6.3). The interaction of year and male plumage ornamentation was

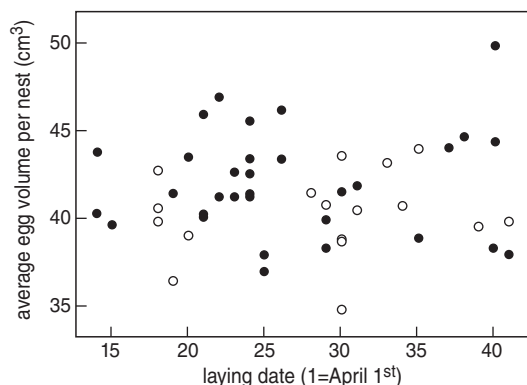


Figure 6.2: Average egg volume per Icelandic black-tailed godwit nest in relation to the laying date of the nest. Full circles depict nests from 1993, open circles nests from 2008.

Table 6.1: Spearman rank correlations between male and female breeding plumage scores of Icelandic black-tailed godwits. Given numbers are Spearman's rho (ρ) and two-tailed significance levels.

| | ρ | P |
|-------------------------------|--------|------|
| orange score ($N = 16$) | 0.24 | 0.19 |
| back score ($N = 16$) | 0.27 | 0.15 |
| breeding feathers ($N = 6$) | 0.49 | 0.17 |
| PC1 ($N = 6$) | 0.30 | 0.28 |

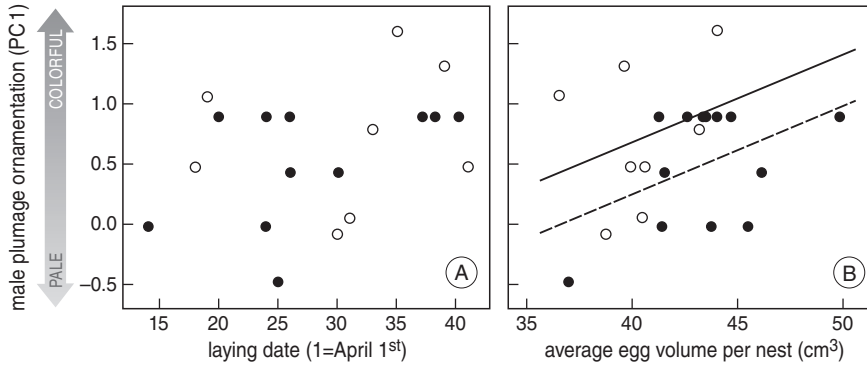


Figure 6.3: Plumage ornamentation (PC1) of male Icelandic black-tailed godwits during breeding season and timing of breeding and the standardized average egg volume of the nest they were incubating. Full circles depict nests from 1993; open circles nests from 2008. Lines are linear regression lines, dashed=1993, dotted=2008. For statistics see text.

removed from the final model (model including the interaction: $\beta_{PC1*year} = -5.45 \pm 6.94SE$, $t = -0.79$; $P = 0.44$; final model including PC1 and year as main effects: $\beta_{PC1} = 4.77 \pm 3.30SE$, $t = 1.45$; $P = 0.17$; $\beta_{year} = 0.24 \pm 0.32SE$, $t = 0.77$; $P = 0.45$, $N = 21$). However, males with a more colorful plumage described by PC1 were mated with females that laid larger eggs (Figure 6.3). The interaction of PC1*year was removed from the final model, indicating that the effect did not differ between the two years (model including the interaction: $\beta_{PC1*year} = -2.23 \pm 2.21SE$, $t = -1.01$; $P = 0.33$; $N = 21$). The variable PC1 was left in the remaining model, and it was significant (Figure 6.3, $\beta_{PC1} = 2.45 \pm 1.12SE$, $t = 2.20$, $P = 0.04$, $\beta_{year} = -0.36 \pm 0.11SE$, $t = -3.30$, $P = 0.004$, $N = 21$), which indicates that in both years, more ornamented males were mated with females that lay larger eggs. Univariate correlation analyses of the raw variables supported this since average egg volume correlated positively with orange score, there were positive trends for the other two scores: more orange colored males were mated with females who laid larger eggs (Table 6.2). We found no relationship between the raw

Table 6.2: Spearman rank-correlations between male plumage scores (orange, back, breeding feathers) of male Icelandic black-tailed godwits during breeding season and egg volume and laying date of the eggs they were incubating. Given numbers are Spearman's rho (ρ) and two-tailed significance levels.

| | average egg volume | | laying date | |
|----------------------------|--------------------|------|-------------|------|
| | ρ | P | ρ | P |
| orange score (N = 29) | 0.42 | 0.02 | -0.10 | 0.62 |
| back score (N = 29) | 0.31 | 0.11 | 0.18 | 0.33 |
| breeding feathers (N = 22) | 0.38 | 0.08 | 0.15 | 0.50 |

male plumage scores and laying date (Table 6.2). We carried out linear regression for each dataset (1993 and 2008) separately, and the relationship between PC1 and egg volume was marginally significant in 1993 ($F_{3,9} = 2.40$, $P = 0.05$, $R^2 = 0.44$, with location as fixed factor), but not in 2008 ($F_{1,6} = 0.57$, $P = 0.47$, $R^2 = 0.09$, only one location).

Discussion

PLUMAGE ORNAMENTATION OF MALE Icelandic black-tailed godwits was statistically significantly related to egg size in 1993, however, the more powerful linear mixed model revealed the pattern for both years, 2008 and 1993 (Figure 6.3). The reason this did not show in the 2008 data in the univariate analysis may be the low sample size, or because we only used a single study area. This is additional, albeit circumstantial support for the interpretation that breeding plumage is a sexually selected signal in black-tailed godwits (Schroeder *et al.*, 2008; 2009).

We use egg volume as an indicator for reproductive success, but since it has been shown to reflect female nutritional status at laying, it may also indicate female reproductive investment (Amat *et al.*, 2001; Sanchez-Lafuente, 2004; Karell *et al.*, 2008). In godwits like in other bird species, female nutritional status is related to the size of the eggs laid (Schroeder *et al.*, 2009). Further, egg volume is related to chick mass at hatching, indicating that egg volume constrains hatchling mass, which affects chick survival in black-tailed godwits (Schekkerman *et al.*, 2008; own data). Godwit chicks are precocial and most other factors influencing the probability of fledging success are stochastic: food quality and availability, adverse weather conditions, predation and agricultural activities (Gill *et al.*, 2007; Schekkerman & Beintema 2007; Schekkerman *et al.*, 2008). Thus one of the few ways a female can influence chick survival before hatching is via the amount of nutrients she allocates to her eggs. Female birds may invest more in reproduction if mated with a high quality mate (Cunningham & Russell, 2000; Loyau *et al.*, 2007). Thus a male godwit incubating a nest that contains relatively large eggs, in our case a more ornamented male has been able to attract a high quality female and or a female willing to invest relatively much into reproduction (Figure 6.3). Since our results are of a correlative nature, this relationship does not necessarily mean female reproductive investment is causally linked to male plumage ornamentation. A female may use other cues as well, like territory quality or nesting density. But our results support that in godwits, male plumage ornamentation and female reproductive investment are directly or indirectly linked and if other cues are involved they can be expected to correlate with male plumage, too.

Black-tailed godwits molt into breeding plumage already on the staging grounds, before engaging the costly spring migration towards Iceland. If molt is costly in terms of energy or time, a more ornamented breeding plumage together with an early arrival could be an honest signal of individual quality, because such a male was able to spend time and nutrients on molt and additionally on early and or fast migration (Piersma & Jukema, 1992; Kokko, 1999; Drent *et al.*, 2003). Despite this, we find no relationship

between timing of breeding and male plumage ornamentation (Figure 6.3). One explanation for this could be that arrival time and timing of breeding may vary between breeding areas of different quality (Gunnarsson *et al.*, 2006b), but less within (high quality) areas. In such a case, male plumage ornamentation would not be expected to vary with timing of laying. Also, we did not find evidence for a seasonal decline of egg volume (Figure 6.2). It may indeed be that in the high quality breeding areas studied in this paper, seasonally changing food availability for adults is not the constraining factor when it comes to reproductive investment decisions of females, and timing for the nutritional requirements for the precocial offspring may play a more important role (Roodbergen & Klok, 2008). It may be worthwhile to study breeding areas of different quality, where food quality and quantity differ (Gunnarsson *et al.*, 2005a).

Contrasting to the nominate race, where paler males do better, we find that more ornamented male Icelandic godwits were mated with females that laid larger eggs, confirming our expectations. In the Icelandic black-tailed godwit, intrasexual competition is most likely higher than in the *limosa* subspecies (Gunnarsson *et al.*, 2005a; -b). If indeed male plumage ornamentation does signal a male's abilities to secure high quality territories, the importance of this information to females may indeed differ between the two subspecies. In Iceland, it may be important to secure a high quality territory, while in The Netherlands, where territory quality with respect to food for adults pre-egg laying is overall high (Bijlsma *et al.*, 2001), this may be less important.

Our evidence is merely correlational and relies on a rather small sample. It may be that Icelandic female godwits base their reproductive investment decisions on plumage traits of her mate, or on the territory quality, or any other male trait that is correlated with a colorful plumage, which in turn could cause the statistical relationship we find. However, because the inverse relationship between egg size and male plumage was found in Dutch godwits, we suggest that this pattern is most likely based on factors that differ between both species and affect plumage ornamentation, with habitat differences and the population growth rate being most pronounced. It suggests that in black-tailed godwits of both subspecies, intrasexual selection is most likely involved in the evolution and maintenance of sexually dimorphic plumage traits.

Acknowledgements

A grant from the Prins Bernard Cultuurfonds to the Foundation Working Group International Waterbird and Wetland Research (WIWO) financed field work in Iceland in 2008 (MR & JS), and a grant from the British Ornithologists Union (BOU) and the Dutch Ministry of Agriculture, Nature Management and Fisheries to the WIWO financed field work in Iceland in 1993 (GG & NG). JS was supported by a start-up grant from the University of Groningen to Theunis Piersma. We thank Jos Hooijmeijer and the Animal Ecology Group of the University of Groningen for kindly letting us use optical equipment on the 2008 trip. Dick Visser helped with creating the figures. Catching and videotaping of birds were undertaken in compliance with Icelandic law. Christiaan Both and Theunis Piersma kindly provided constructive comments on the manuscript. Tomas G. Gunnarsson helped us with logistics in Iceland and introduced us to the most important godwit breeding sites. We thank Glenn Kocak, Hans de Waard, Francisco Santiago Quesada, Domnic Cimiotti and Dagmar Kudernatsch for enthusiastic data collection and enjoyable company.

Seven

Degree of melanin plumage coloration in a grassland-breeding shorebird: interpretable correlations with nest density

Julia Schroeder, Niko M. Groen, Rosemarie Kentie, Pedro M. Lourenço,
Jos C. E. W. Hooijmeijer, Christiaan Both, Theunis Piersma

Abstract

Sexually selected male plumage ornamentation is known to play a role in male-male interactions about territory acquisition. Melanin plumage ornamentation has also been proposed to show pleiotropical links with steroid levels and aggressiveness. Here we present evidence consistent with a link between melanin plumage coloration and territorial behaviour, the latter integrated as nest density, in a grassland breeding shorebird, the black-tailed godwit *Limosa limosa*. Paler male godwits nested at higher densities, their nests were defended by a larger number of birds and their eggs had a higher probability of hatching. Nests at higher densities contained larger eggs. A previous study showed that paler males were mated with females that laid larger eggs, and our data are consistent with this even when nest density was statistically accounted for. To explain why paler birds may nowadays have an advantage over more colourful birds, we argue that a recent change of habitat quality and heterogeneity may have led to relaxed competition for nest sites. We suggest that females in areas with an abundance of high quality habitat choose for either pale males or high nest density sites, because these males tolerate nesting at higher densities where fitness is predictably higher. Thus, male melanin plumage coloration may also be linked with territorial behaviour, with historically changing plumage ornamentation patterns as the phenotypic outcome.

Introduction

SEXUALLY SELECTED PLUMAGE ORNAMENTATION can evolve when conspicuous plumage ornaments signal competing abilities to conspecifics and when individuals compete for scarce resources such as mates, access to food, or nesting sites (Andersson, 1994). If competitors of higher quality are preferentially mated with, and sire more, offspring, and when ornamentation is heritable, this may lead to exaggerated and conspicuous plumage ornamentation (Andersson, 1994) that must be costly in order to be reliable (Grafen, 1990). Until recently, it was unknown how the expression of melanin-based plumage ornaments could evoke physiological costs (McGraw *et al.*, 2002; McGraw 2008; but see Griffith *et al.*, 2006). Ducrest *et al.*, (2008) proposed that the POMC gene pleiotropically links melanin coloration, stress-resistance, aggressiveness and steroid levels. Since coping with stress and steroid receptiveness may vary between individuals, such genetic linkage provides an explanation for the costs of melanin plumage coloration (Kempnaers *et al.*, 2008). Further, these behavioural traits and melanin coloration may be linked by dynamic feedbacks rather than being unidirectional (Rubenstein & Hauber, 2008). This suggests, without assuming directed causality, that more ornamented individuals would be more aggressive, have higher stress-resistance and steroid levels, and more likely to succeed at competitive interactions (McGraw *et al.*, 2003). The feedback dynamics may also explain empirical correlations between sexually selected plumage traits and environmental changes or social interactions, and thus orchestrate the evolution and maintenance of sexually selected plumage coloration (Safran *et al.*, 2008; Rubenstein & Hauber, 2008).

Black-tailed godwits (*Limosa l. limosa*) provide an example of a species in which sexually selected, melanin plumage coloration has undergone historical change at the population level (Schroeder *et al.*, 2008; 2009), and of which individuals show covariation between POMC gene variation and plumage (A. Roulin *et al.*, unpubl.; Toral *et al.*, 2008). Male black-tailed godwits defend their territory by ground and aerial displays where the melanin plumage may be important (Lind, 1961; Green *et al.*, 1990; Beintema *et al.*, 1995). If in godwits plumage coloration and aggressiveness were indeed pleiotropically linked, we would expect male godwits with a more colourful plumage to be more successful in competitive interactions with conspecifics (McGraw *et al.*, 2003) and being able to secure a larger territory of (in heterogeneous habitats) presumably better quality (Fretwell & Lucas, 1970).

However, for a vulnerable open-nesting species like black-tailed godwits, breeding in close proximity to conspecifics can also be beneficial, for example because it enables group defence strategies (Green *et al.*, 1990; Beintema *et al.*, 1995; MacDonald & Bolton, 2008). Nest defence or anti-predator behaviour to protect the clutch and the offspring is common among birds (e.g. Kis *et al.*, 2000; Kontiainen *et al.*, 2009). High nesting density can reduce predation risk, because the risk of being injured or killed during predator defence is spread among many, and because more defending birds are more effective in driving predators away (Inman & Krebs, 1987; Kis *et al.*, 2000).

Here we examine the following predictions for black-tailed godwits breeding on a high-quality breeding site in The Netherlands. (1) We expect that at higher nest densities, more godwits will be involved in predator defence. (2) In homogenous habitats of relatively high quality like hay meadows in The Netherlands, nests that are defended by more birds should contain larger eggs and be more likely to hatch than more solitary nests. (3) We expect more ornamented godwit males to breed at lower densities than less ornamented males. Size may enhance agility, and thus effectiveness of aerial defence; therefore, we additionally analyzed breeding density with respect to wing length (Jönsson & Alerstam, 1990).

Methods

Field methods

We carried out our studies at the Northern part of the Workumerbinnenwaard, province of Friesland, the Netherlands (52°59'N, 5°24'E) in 2004–2007. This is an area of 243 ha grassland mown from early June onwards. For a detailed description of the study area see Van den Brink *et al.*, (2008). Briefly, local volunteers of the meadow bird conservation group Fûgelwacht Warkum found nests and reported approximate locations to us. We revisited the nests and determined exact positions with handheld Garmin GPS 12 devices to the nearest 2 m. We calculated nest density as the number of all nests in the area of 250 m radius around the focal nest (hereafter “density”). We chose a radius of 250 meters because this is a reasonable indication of a godwit territory (Groen, 1993; Van den Brink *et al.*, 2008). To calculate density, we excluded all nests that were located on edges to areas that harboured godwit nests in unknown numbers. However, these nests were still used for calculation of density for other nests.

At the first visit, eggs were measured. Incubation stage and estimated hatching dates were calculated following Liebezeit *et al.*, (2007). Average egg volume was calculated by the formula $(\text{length} \times \text{width}^2 \times 0.52)$ and averaged per nest (Romanoff & Romanoff, 1949). Egg volume did not change over the course of the season; however, it may vary between years (unpubl. data). Therefore, we standardized egg volume per year. To minimize disturbance, nests were only visited again shortly before predicted hatching, to confirm hatching success (yes/no) and to catch adults (see below). This resulted in 323 nests with known nest density, egg volume and hatching success.

From 2005 onward, we counted the number of godwits alarming in flight when we approached a nest (Brunton, 1990). Because birds often do not defend nests early in incubation and during egg laying (Kis *et al.*, 2000; pers. obs.), we only used data of the first observation of nests incubated for more than 10 and less than 20 days. We have data on the numbers of alarming birds, hatching success and density near 143 nests from 2005–2007.

Adult godwits were captured in late incubation in 2004–2007. When cracks were found in the eggs three days before the estimated hatching date, or when the chicks were audibly beeping from inside the eggs, two indicators of a very close hatching

date, catching attempts were undertaken with either a walk-in trap or an automatic fall-trap. The traps were constantly monitored by an observer from a hidden place (ditch edge). Immediately after a bird entered a walk-in trap and sat down on its nest, an observer started running towards it causing the bird to flee. The funnel shaped entrance of the walk-in trap prevented the bird from escape until the observer reached the cage. The automatic fall-trap consists of two metal rings connected with mistnet fabric. Both rings rest atop of three metal poles that are placed around a nest. This construction allowed a bird to enter the trap from all sides. Once the bird sat down on its nest, the lower ring was released by a remote control and the bird was trapped, and immediately picked up by an observer. The two types of traps did not lead to any observable change in the birds behaviour and we never observed nest abandonment after catching attempts (Schroeder *et al.*, 2008; 2009).

A blood sample of 20 μ l was taken from the brachial vein for molecular sex assignment, for which we followed the protocol described as in Griffiths *et al.*, (1998), slightly modified to prevent sexing errors following Schroeder *et al.*, (2008). We measured wing length (flattened and straightened, ± 1 mm). We took digital pictures of the birds in a standardized way and scored breeding plumage of all male birds (black bars on the belly, the orange plumage on neck and breast, the amount of white plumage in the head plumage, the extend and number of breeding feathers on the back), as detailed by Schroeder *et al.*, (2008; 2009). We collapsed the plumage scores in a principal component analysis. The first principal component (PC1 hereafter) explained 53.6% of variation in plumage coloration, and birds that score high are more colourful than birds that score low (eigenvalue: 2.68). This resulted on plumage, wing length and density data on 56 observations of 51 male godwits. Variation in sample sizes results from missing values. All fieldwork was carried out in accordance with the Dutch Animal Welfare Act Article 9, and the Animal Welfare Office of the University of Groningen granted permission to conduct this work under the under licence number DEC 4112B.

Statistics

We tested whether aerial defence was related with a higher hatching success and whether nests defended by a larger number of godwits contained larger eggs. If, because of the increasing value over time of the clutch, predator defence were stronger later during incubation, we would expect late nests to be defended by more birds. Since nests late in incubation would have a higher probability to hatch than nests found earlier, this gives a potential bias (Mayfield, 1975). We accounted for this bias by using only the first observation of each nest after the 10th day of incubation and adding the incubation stage as a covariate to the models. We tested for the effect of defence on hatching success with a generalized linear model (GLM) with a binomial error distribution and a logit link function. To test for an effect of defence on egg volume we used a linear model (LM). We then tested whether nests in higher densities were defended by more godwits than nests in lower densities, and we used a GLM with a Poisson error distribution and a log link function, adding year as a factor and incubation stage as a covariate.

To investigate whether male plumage and wing length influenced breeding density, we used a generalized linear mixed model (GLMM), with a Poisson error distribution and a log link function, where individual males were modelled as random effects on the intercept to correct for pseudoreplication (Gelman & Hill, 2006). We tested for quadratic effects of wing length and plumage coloration. Females may choose to lay larger eggs when mated with a more colourful male, when nesting at high nest densities, or both. We used a linear mixed model (LMM) to explain standardized egg volume, with plumage and density as explanatory covariates, and individual as random effect. We used a GLM with a binomial error distribution and a logit link function to determine whether nests in higher densities would be more likely to hatch, and added year as fixed factor. There was no evidence for overdispersion in any of our models.

We used R 2.8.1 for computing statistics, the `glm()` function for computing GLMs, the `lm()` function for LMs and the `lmer()` function of the `lme4` package to compute LMMs and GLMMs (R Development Core Team, 2008). We report parameter estimates and confidence intervals of the most parsimonious model obtained using the Akaike information criterion (AIC, Burnham & Anderson, 2002).

Results

THE BINOMIAL LOGISTIC MODEL explaining hatching success showed that nests with more birds taking part in the aerial defence were more likely to hatch (Fig. 7.1; GLM, $b_{\text{defence}} \pm \text{SE} = 0.56 \pm 0.19$, $Z_{140,142} = 2.93$, $P = 0.003$, $N = 143$). The covariate ‘incubation stage’ was not significant, but we left it in the final model to correct for the bias in hatching success ($b_{\text{incubation stage}} \pm \text{SE} = -0.06 \pm 0.07$, $Z_{140,142} = -0.82$, $P = 0.41$). Nests that were defended by a larger number of godwits did not contain larger eggs (LM: $F_{1,137} = 0.61$, $P = 0.44$; $N = 139$). Nests at higher densities were defended by a larger number of birds (GLM, final model: $b_{\text{defence}} \pm \text{SE} = 0.05 \pm 0.21$, $Z_{139,142} = 2.07$, $P = 0.04$, $N = 143$). The covariate ‘incubation stage’ did not contribute significantly to the variation here either, but like the variable year, we left it in the final model to correct for any bias in hatching success ($P_{\text{incubation stage}} = 0.33$, $Z_{139,142} = -0.98$, $P_{\text{year}} < 0.001$, $Z_{139,142} = -9.14$). The quadratic term for number of defending birds was not significant and we removed it from the final model (term removed from GLM: $P_{\text{defence}^2} = 0.45$, $Z_{139,142} = 0.76$).

Paler males bred in higher densities than more ornamented males (Fig. 7.2). The quadratic term for wing length and wing length itself were removed from the final model (GLMM including the quadratic term wing length^2 compared with the final model: $P_{\text{wing length}^2} = 0.77$, $\text{dAIC} = 60$; $P_{\text{wing length}} = 0.96$, $\text{dAIC} = 2.0$). Similarly, the quadratic term of plumage coloration was not statistically significantly related to breeding density and we removed it from the final model ($P_{\text{PC1}^2} = 0.35$, $\text{dAIC} = 1.3$), which then only contained the variable for plumage coloration (final model GLMM: $b_{\text{PC1}} \pm \text{SE} = -0.18 \pm 0.08$, $Z = -2.18$, $P = 0.02$; $N = 56$ observations of 51 individuals).

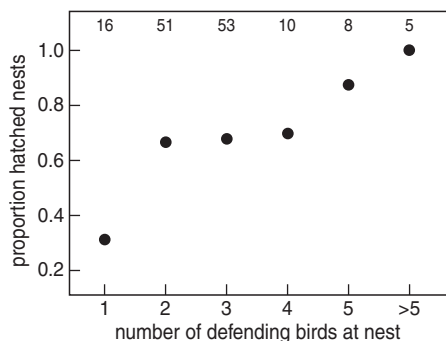


Figure 7.1: The proportion of successfully hatched nests in relation to the number of godwits alarming when an intruder (researcher) made a nest control at black-tailed godwits nest in The Netherlands. Numbers above the graph represent sample sizes.

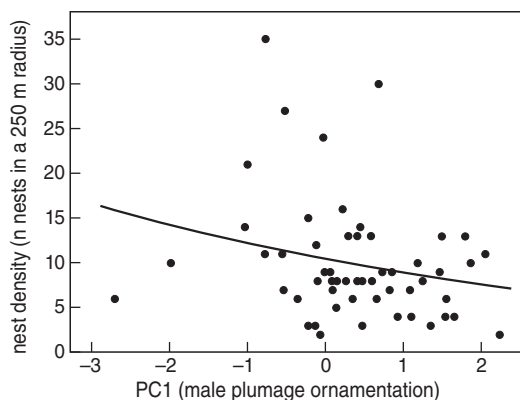


Figure 7.2: Plumage ornamentation of male black-tailed godwits (measured as PC1, higher scores translate to more colourful birds) in relation to nest density (number of nests in a radius of 250 meters around the focal nests) of godwits breeding in The Netherlands. Line depicts model prediction of a GLM with a Poisson error distribution and log link function, density as response and PC1 as explanatory variable.

We found that nests in higher density areas contained larger eggs (Fig. 7.3; LM: $b \pm SE = 0.02 \pm 0.009$, $F_{1,321} = 3.96$, $P = 0.047$). We found no effect of density on hatching success (GLM, $b \pm SE = -0.01 \pm 0.02$, $Z_{321,322} = -0.47$, $P = 0.64$; $N = 157$ hatched, 166 did not hatch). Year did not remain in the most parsimonious model ($dAIC = 2.11$).

In a model explaining egg volume on the basis of nest density and male plumage ornamentation, in contrast to the result presented above, the parameter estimate for nest density was not significant ($P = 0.59$) and unlike male plumage ornamentation, it was removed from the final model ($dAIC = 7.72$). There was a tendency for paler males to be mated with females laying larger eggs, although the parameter estimate

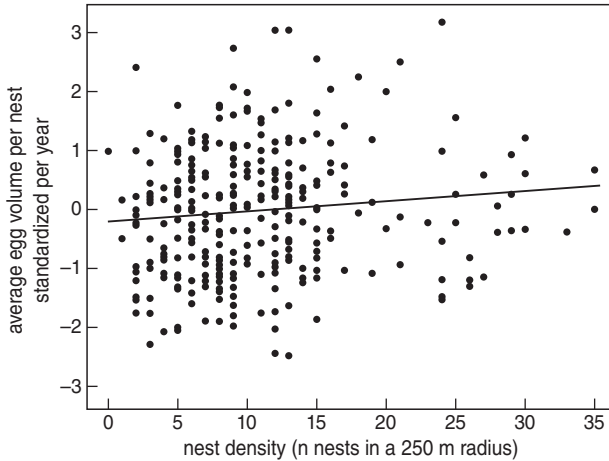


Figure 7.3: Nest density (number of nests in a radius of 250 meters around the focal nests) of Black-tailed godwits breeding in The Netherlands in relation to average egg volume per nest, standardized per year. Line represents linear regression.

itself was not significant (LMM: $b \pm SE$: -0.29 ± 0.14 ; $t = -2.03$, $P = 0.14$; $N = 53$ observations on 49 males). It must be noted that within a larger sample in the same dataset (due to males caught on nests that were considered edge nests here, see methods), paler males were statistically significantly mated with females laying larger eggs (Schroeder *et al.*, 2009).

Discussion

WE FOUND CORRELATIONS BETWEEN NEST DEFENCE or alarm behaviour, nest density, male plumage ornamentation and reproductive parameters in a Dutch black-tailed godwit breeding population. Our results support the notion that these behaviours are linked, and that these links may play a role in the maintenance of sexually selected traits in black-tailed godwits.

Nests at higher densities were defended by more godwits, and contained larger eggs than more solitary nests. Further, nests defended by a larger number of godwits were more likely to hatch. Increasing density can lead to decreasing fitness expectations, but it can also be beneficial (Fretwell & Lucas, 1970). Predators seem to avoid, or be less successful in, areas that are defended by many birds in contrast to areas with little defence (Elliot, 1985; Inman & Krebs, 1987). More birds defending together against predators is often related with higher nesting success (e.g. Becker, 1995; Larsen *et al.*, 1996; Harris, 2008; MacDonald & Bolton, 2008). Usually, and this is not different in our study area, godwits breed close to other meadowbirds sporting a similar defence strategy against aerial predators, like Northern lapwing (*Vanellus vanellus*) and

Eurasian oystercatcher (*Haematopus ostralegus*, Beintema *et al.*, 1995). These birds, when nesting close by, will help driving predators away. Not including these shorebird species in our analysis may have lowered our statistical power but should not affect the direction of our results. Also, we cannot fully exclude the possibility that godwits simply breed at higher densities in areas with higher breeding success and/or lower predation pressure. However, we consider this unlikely because we measured density on a relatively small scale (250m around the nest), whereas the breeding area most likely has a spatially rather constant level of predation pressure from birds of prey. Therefore, we suggest that anti-predation behaviour of black-tailed godwits may be more effective in preventing clutches from being depredated when more godwits participate.

We have shown that females lay larger eggs in areas with higher nest density. Yet, when male plumage was added to the analysis in a smaller sample size, the factor nest density was no longer significant. Since egg production is physiologically costly for females, in species with an invariant clutch size like black-tailed godwits egg volume may be an indication of female reproductive investment (Williams, 2005; Bonato *et al.*, 2009). A female may decide how much to invest in current reproduction, i.e. the size of the eggs to produce, dependent on certain cues, for instance territory quality or mate quality (Cunningham & Russell, 2000; Loyau *et al.*, 2007). Two possible cues are nest density and/or male plumage ornamentation. In our relatively small dataset, this relationship was best explained with male plumage as found before (Schroeder *et al.*, 2009). Female godwits lay larger eggs when mated with paler males who apparently happen to breed at higher densities. That the effect in the present study is not statistically significant most likely reflects lack of statistical power.

In any case, this correlation between male plumage and female reproductive investment does not necessarily mean that the two are causally related. Such a correlation could also arise if females adjust reproductive investment according to the quality of the nesting site, which they may assess, possibly next to nest density, by additional features like estimated food resources for offspring, prior breeding success at a given nest location, or male timing of migration. The latter could be reflected by male breeding plumage (Piersma & Jukema, 1993; Schroeder *et al.*, 2009). Although we cannot conclude which cue (plumage or density) may be more important (Schroeder *et al.*, 2009), we think it is important that the two traits were suggested to be pleiotropically linked by the POMC gene and melanocortin receptors (McGraw *et al.*, 2003; Ducrest *et al.*, 2008; Rubenstein & Hauber, 2008). This supports the suggestion that both, intersexual competition for territories and male plumage play a role in sexually selected traits in godwits.

Paler males bred in higher densities. Thus, more ornamented male godwits were able to evict other godwits from their territory, or paler males preferred or were enabled to nest in closer proximity to conspecifics. Johnson & Lanyon, (2000) suggested that greater habitat heterogeneity in marshes would lead to stronger inter-male competition, and favour the evolution of sexually selected ornaments. Turning around their argument, in godwits, a historical habitat change from marshes to

meadows may have lead to relaxed intersexual competition for nesting sites, due to the higher food abundance for adults in agricultural areas (Haverschmidt, 1963; Bijlsma *et al.*, 2001; Schroeder *et al.*, 2009). This may have lead to signalling in male-male competition become less important and allowed birds to nest closer together. Further, predation danger increased, while breeding success decreased during the last decades (Schekkerman *et al.*, 2009), and both factors may lead to cooperative defence and breeding in higher densities become more important.

Conclusion

Our data are consistent with the idea that in black-tailed godwits, male plumage ornamentation is linked with behaviour, and that the interplay between melanin ornamentation, nesting density and predator defence behaviour may be important for the evolution and maintenance of sexually selected traits in godwits.

Acknowledgements

Petra de Goeij, Valentijn van den Brink, Freek Mandema and all the students involved in the fieldwork deserve credit here. We thank the conservation authority It Fryske Gea and the local farmers for their co-operation and for allowing access to their grounds. The assistance of the local volunteers of the Fûgelwacht Warkum for searching nests and reporting their locations to us has been invaluable. Alex Roulin provided constructive advise on the manuscript. This work was financially supported by a set-up grant to T.P. from the University of Groningen, grants to J.S. from the Schure-Beijerinck-Popping foundation and by BirdLife-Netherlands. P.M.L. was supported by a grant from the Portuguese Science and Technology Foundation.

Box C

Effects of diet

Julia Schroeder, Francisco Santiago-Quesada, José A Masero,
Juan M Sánchez-Guzmán, Christiaan Both & Theunis Piersma

Introduction

BLACK-TAILED GODWITS *Limosa l. limosa* are temperate breeding migratory waders (Beintema *et al.*, 1995). Their wintering areas lie in West Africa (Lourenço & Piersma, 2008b). From December onwards, godwits start their northward migration to the breeding grounds. Large numbers of black-tailed godwits of the nominate race arrive in Spain and Portugal on the rice fields in late December and early January, where they forage on rice seeds (Sánchez-Guzmán *et al.*, 2007; Lourenço & Piersma, 2008a). A small part of the population does not migrate via South-western Europe, but uses stop-over sites in Tunisia, Morocco, France, Greece and Italy, where invertebrates are most likely their main prey items (Kuijper *et al.*, 2006, Lourenço & Piersma, 2008b). From here on, the last part of spring migration continues, and the first birds arrive in their Dutch breeding areas in the beginning of March (Chapter 2). During the breeding season, godwits forage mainly on earthworms and tipulid larvae. However, when, during dry periods, the soil becomes too hard to be penetrated, godwits are also known to feed on small insects living in the grass strata (Beintema *et al.*, 1995).

Not much is known about how black-tailed godwits cope with this variety of diet. In this box, we take the first step and examine how different diets affect body mass gain and breeding plumage development of black-tailed godwits during their last stop-over before reaching the breeding grounds.

Methods

THE LAST MAJOR STOP-OVER sites before the breeding grounds for black-tailed godwits breeding in The Netherlands are the rice fields of the Iberian Peninsula (Lourenço & Piersma, 2008a, Sánchez-Guzmán *et al.*, 2007). Godwits spend here nearly two month before continuing their journey to the breeding grounds (F. Santiago Quesada & J. A. Masero, pers. comm.). This is where they molt into breeding plumage (per. obs.). Godwits were caught on the rice fields in Iberia close to the village Hernán Cortéz in Extremadura, Spain (30°01'N, 5°55'W, for a detailed description of the study area see Sánchez-Guzmán *et al.*, 2007). These birds were weighed on capture, and a blood sample was taken for molecular sexing and genotyping with respect to CHD1-Z (Chapters 4, 8). One male in the rice group was found to be of the rare Z* genotype. Birds were housed in large outdoor cages (5 × 2.5 × 2 m), provided with fresh water *ad libitum* and grit for gastroliths.

We created two groups. The first group consisted of two cages with 6 male and 5 female godwits that were fed fly larvae *ad libitum*. The second group consisted of two cages with 7 male and 5 female godwits fed rice *ad libitum*. For the first week, birds were fed a mixed diet of rice and fly larvae for a week to habituate to captivity and feeding trays. Fly larvae were bought at a local store selling equipment for fishermen. The rice came from the rice fields in Extremadura that godwits use for staging. The treatment took place for five weeks, during which all birds were weight weekly to the

nearest gram. On the same occasions, digital pictures were taken of the birds. Because birds would not moult into a full breeding plumage over the duration of the experiment and to verify that it would model the real world to a satisfactory degree, we took another set of digital photos from all experimental birds 6 weeks after the experiment was finished, assuming that by then the breeding plumage was fully expressed. Excluding this data did not change the outcome of our analyses.

We used the plumage scores described in Chapter 4. White in head, white in neck and black in neck could not easily be distinguished from the predominant grey winter plumage. We therefore decided to only use scores that changed noticeably over the course of the experiment, and that were orange, bars, back and feathers. These scores were collapsed in a principle component analysis that resulted in one component with an eigenvalue of 2.77. This principal component explained 72% of the variation in plumage ornamentation. Birds that score high on PC1 were more colorful with respect to all plumage scores than those with a lower PC1 score.

We calculated body mass gain as the difference between body mass at the start of the treatment (after a week of habituation) and body mass after four weeks of treatment. To test whether body mass gain differed between the treatments, we employed a linear mixed model, with treatment as fixed factor and cage as random effect. Since diet may have a different effect on the sexes (Santiago-Quesada *et al.*, 2009), we included sex (females were coded as 0, males as 1) as a fixed factor and the interaction between sex and treatment in the model. Genotype (CHD1-Z) affects female, but not male body condition (Chapter 8). Since we only had one bird of the rare genotype we ignored it in the main analysis and ran another one on the dataset with that male excluded.

We analyzed plumage (PC1) as dependent variable in a linear mixed model (LMM); individuals nested in cages were modeled as random effects, treatment and sex as fixed factors and week as covariate. We expected godwits to get more colorful over time. Further, it may be that the sexes differ in their reaction to the treatments (Chapter 9). Therefore, we also tested for the two-way interactions between week and treatment and sex and treatment. We coded females as 0 and males as 1. Alike, fly larvae diet was used as the reference category. Since we know that genotype (CHD1-Z) affects plumage expression in males (Chapters 4, 8), we again ran two models, one with that male in the dataset, and an additional model with it excluded. We used R and the `lme()` function of the `nlme` package to compute LMMs (R Development Core Team 2008). We selected the most parsimonious model by AIC (Burnham & Anderson, 2002).

Results

ALL CAPTIVE BLACK-TAILED GODWITS LOST BODY MASS during the first week of habituating to captivity (Fig. C.1A, B). Since we were only interested in the effect of diet, we excluded the measurements from the day of capture for the analysis of body

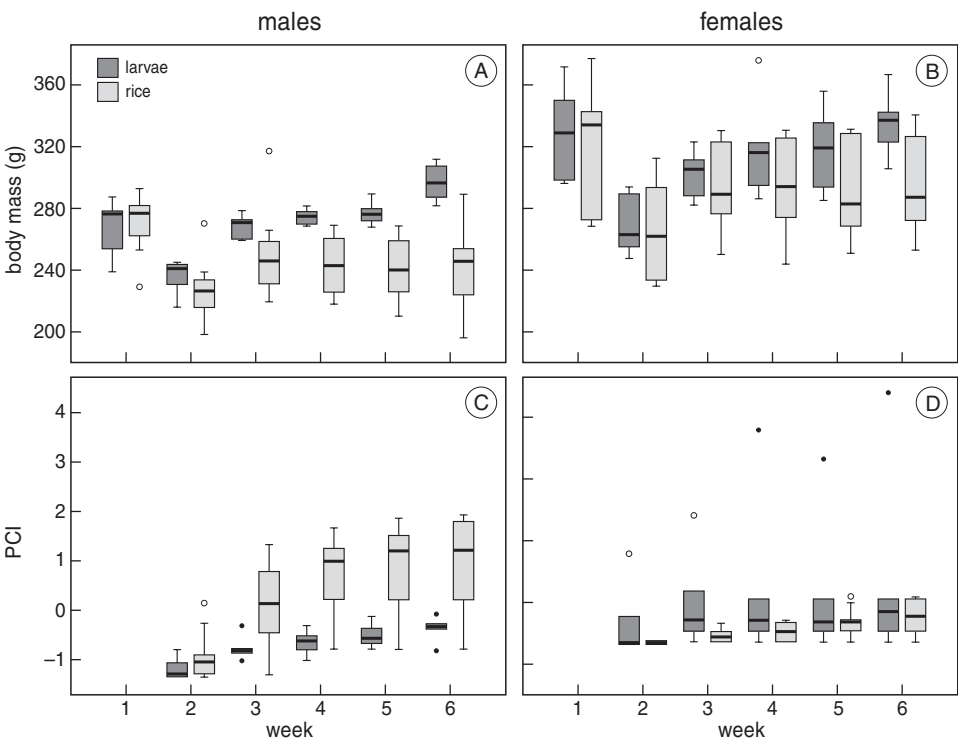


Figure C.1: The effect of diet (rice and fly larvae) on body mass (A, B) and plumage ornamentation (PC1, C, D) in male and female black-tailed godwits in captivity. Week 1 represents measurements taken at capture, treatment started after measurements were taken at week 2.

Table C.1: Results of linear mixed models on the effect of a diet (fly larvae or rice grains) on plumage ornamentation (PC1) in captive black-tailed godwits. Observations (N = 132/126) on individual birds (N = 22/21) nested in cages (N = 4) were modeled as random effects. Female was the reference category for sex, fly larvae for treatment.

| Effects in the final model | dataset | | | | | |
|----------------------------|-------------|-------|--------|-------------|-------|--------|
| | full | | | without Z* | | |
| | estimate±SE | t | P | estimate±SE | t | P |
| Week | 0.15 ±0.02 | 6.61 | <0.001 | 0.15 ±0.02 | 6.56 | <0.001 |
| Treatment (rice) | -0.11 ±0.52 | -0.20 | 0.86 | -0.08 ±0.46 | -0.17 | 0.88 |
| Sex (male) | 0.88 ±0.29 | 3.09 | 0.007 | 0.98 ±0.30 | 3.27 | 0.005 |
| Week x treatment | 0.06 ±0.03 | 1.78 | 0.08 | 0.07 ±0.03 | 2.07 | 0.04 |
| Rejected effect | | | | | | |
| Treatment x sex | 0.22 ±0.59 | 0.37 | 0.72 | 0.49 ±0.61 | 0.80 | 0.43 |

mass change. We found that birds feeding on rice did not fatten up as the birds that were fed fly larvae did (Fig. C.1A, B). Total body mass gain in the rice group was $30.8\text{g} \pm 6.84\text{SE}$ in females and $6.17\text{g} \pm 8.78\text{SE}$ in males. In the fly larvae group, females gained on average $69.8\text{g} \pm 9.23\text{SE}$, males $59.33\text{g} \pm 2.96\text{SE}$. This difference was significant between treatments and between sexes (LMM, fly larvae and female as reference groups: $\beta_{\text{rice}} = -46.73 \pm 7.25$, $t_{\text{treatment}} = -6.49$, $P_{\text{treatment}} = 0.02$; $\beta_{\text{males}} = -17.56 \pm 7.28$, $t_{\text{sex}} = -2.41$, $P_{\text{sex}} = 0.03$; $N = 22$ observations in 4 groups). We removed the interaction between treatment and sex from the most parsimonious model ($\beta_{\text{rice} \times \text{males}} = -13.18 \pm 14.36$, $t_{\text{treatment} \times \text{sex}} = -0.92$, $P_{\text{treatment} \times \text{sex}} = 0.37$). The results did not change quantitatively, and only little qualitatively, when excluding the Z* male (same model structure: $\beta_{\text{rice}} = -48.67 \pm 7.35$, $t_{\text{treatment}} = -6.62$, $P_{\text{treatment}} = 0.02$; $\beta_{\text{males}} = -19.33 \pm 7.35$, $t_{\text{sex}} = -2.63$, $P_{\text{sex}} = 0.02$; $N = 21$ observations in 4 groups).

Godwits of both sexes became more colorful over the course of time; this was more pronounced in males (Fig. C.1C, D; Table C.1). When excluding the Z* male, we found that the birds feeding on rice molted a more colorful and ornamented breeding plumage than the birds feeding on fly larvae (Fig. C.1C, D; Table C.1). For both datasets, we removed the interaction between treatment and sex from the most parsimonious model (Table C.1).

Conclusion

WE FOUND THAT, DURING SPRING STOP-OVER, captive black-tailed godwits feeding on fly larvae gained more body mass and molted a less ornamented and colorful breeding plumage than godwits feeding on rice. The little gain of body mass in the rice groups was surprising, since rice is the major diet of black-tailed godwits for up to two month during spring stop-over in Iberia before they engage on a 3000km flight to their breeding grounds (Sánchez-Guzmán *et al.*, 2007; Lourenço & Piersma, 2008a). Further, godwits caught on the rice fields in Extremadura during spring stop-over did not exhibit conspicuously low body masses (own observations).

One study recently analyzed godwit faeces collected on rice fields in Portugal, when godwits were foraging there (Lourenço & Piersma, 2008a). Here, rice grains were the most common food representing 94% of all identified prey items (Lourenço & Piersma, 2008a). Santiago-Quesada *et al.* (2009) found a high assimilation efficiency of rice grains in captive black-tailed godwits (90.0%), and this, together with the energetic value of the food indicates that rice *ad libitum* should be more than sufficient for godwits to fatten up in captivity (P. Lourenço, pers. comm.).

Only a few anecdotal studies describe the effects of plant eating in black-tailed godwits. Lange (1968) noted that his two birds would start eating grains after five days of captivity, preferring them above offered invertebrates. A stomach analysis of godwits from Hungarian rice fields showed a large fraction of rice, but one week later, when the rice fields were dried out, stomachs of godwits caught from the same area contained mainly chironomidae larvae (Sterbetz, 1962). One study on stomach

contents of godwits from Kazakhstan reports from a situation without rice fields, where, even though 58% of the collected stomachs contained grains, the majority of prey items (>70%) in terms of mass and volume were arthropods and insects (Rjabow & Mosalowa, 1967; for an overview see Glutz von Blotzheim *et al.*, 1985 and references within). M. Kersten (pers. comm.) showed that, when forced to switch diets between two different kinds of animal food sources, black-tailed godwits directly lost weight and it took them several days to adjust to the new diet.

In our experiments, females were less affected and gained half of what females gained feeding on fly larvae, while males feeding on rice gained only a tenth of what they gained when feeding on animal prey. One explanation for this could be the sexual size dimorphism; larger females with longer and larger intestines may be better in digesting plant material than males (Santiago-Quesada *et al.*, 2009). However, the difference in body mass gain of birds feeding on rice between the sexes is larger than expected, based on a small difference in assimilation efficiency (91.4% in females, 88.8% in males, Santiago-Quesada *et al.*, 2009). Another explanation may be found in behavioral differences between males that were fed rice and those fed animal prey items. It could be that godwits that were fed rice, and especially males, participated in activities that are costly energy and time wise. We noted that birds in the rice groups were more aggressive towards each other, defending the food trays against each other. It may be that more colorful and ornamented males were more prone to participate in aggressive interactions (McGraw *et al.*, 2003), which in turn may have prevented birds from feeding, which may have led to them gaining less mass than birds feeding on fly larvae (see Chapter 10 for an elaboration of this idea).

This explanation is not sufficient, however, because we do not know why the birds feeding on fly larvae, being in excellent body condition, did not molt into a more colorful breeding plumage, while the birds from the rice group did so. One could argue that birds that are more aggressive towards each other may have higher levels of circulating steroids (i.e. testosterone), which in turn may affect the expression of sexually selected breeding plumage (see also Chapter 7 and 10; Rubenstein & Hauber, 2008). However, this is a circular argument, since we do not know the initial trigger. It may be that the low body mass triggered monopolizing food behavior and exhibiting aggressiveness which in turn may, via an endocrine pathway, led to a more ornamented plumage (Safran *et al.*, 2008). But it also may be the other way around (Safran *et al.*, 2008, Rubenstein & Hauber, 2008). We need more experiments, preferably where testosterone levels and aggressive interactions are quantified, to solve this problem.

In conclusion, although we could not unravel the mechanism, our experiment suggests that diet choice during spring migration is related to body mass gain and plumage acquisition in black-tailed godwit. There also may be a link between plumage and body mass gain in the Black-tailed godwit that deserves our future attention.

Eight

Linking intronic polymorphism on the CHD1-Z gene with fitness correlates in black-tailed godwits

Julia Schroeder, Rosemarie Kentie, Marco van der Velde,
Jos C.E.W. Hooijmeijer, Christiaan Both, Oliver Haddrath,
Allen J. Baker & Theunis Piersma

Abstract

We report an intronic length polymorphism in the CHD1-Z gene in Black-tailed Godwits (*Limosa limosa limosa*). The Z^* allele was found in 14% of 251 adult birds from nature reserves, while Z^* was not found among 33 birds breeding in intensively managed agricultural lands. Males and females with the Z^* allele expressed less breeding plumage, had higher body mass, bred earlier and had bigger eggs. There were no significant differences in annual survival. DNA harvested from museum skins showed that this polymorphism was present at low frequency in 1929. Strong asymmetrical overdominance may explain the low frequency of the Z^* allele. Genetic linkage to causal genes might be an explanation for the phenotypic correlations. Our findings suggest a degree of cryptic genetic population structuring in the Dutch godwit population.

Introduction

MOLECULAR METHODS OF AVIAN SEX ASSIGNMENT make use of intronic DNA (Griffiths *et al.*, 1996; Ellegren & Sheldon, 1997; Griffiths *et al.*, 1998; Fridolfsson & Ellegren, 1999). In birds, males are the homogametic sex (ZZ), while females are heterogametic (ZW). The sexing methods use PCR amplification of a noncoding, supposedly neutral fragment of an intron on the conservative CHD1 gene located on both sex chromosomes, labelled CHD1-Z and CHD1-W, which conveniently differ in base pair length. Males have two fragments of the same length (ZZ genotype), whereas females have two fragments of unequal length (genotype ZW).

However, studies on five auklet species, one rail and three shorebird species report length variation in this locus. In some cases, this complicates band interpretation and can lead to wrong sex assignment (Dawson *et al.*, 2001; Lee & Griffiths, 2003; Robertson & Gemmel, 2006; Schroeder *et al.*, 2008a; Casey *et al.*, 2009; AJB, unpublished data). One study examines fitness correlates of this polymorphism: in Moorhens (*Gallinula chloropus*), Lee *et al.*, (2002) reported increased mortality in male chicks with the polymorphism on CHD1-Z. The authors proposed that CHD1-Z may have hitchhiked with the causal gene(s). In Black-tailed Godwits (*Limosa limosa limosa*), PCR products originating from the Z chromosome are either 374 (the rare type CHD1-Z*) or 378 (CHD1-Z) basepairs (bp) in length. Male godwits could come in three genotypes: 378/378 bp (CHD1-Z/CHD1-Z, hereafter abbreviated as ZZ), 378/374 bp (ZZ*), or 374/374 bp (Z*Z*). The PCR product of the W chromosome is 393 bp long and females could have two different genotypes: 378/393 bp (ZW) or 374/393 bp (Z*W). Schroeder *et al.*, (2008a) found 29% of 70 sexed male godwits to be of genotype ZZ*, none had the Z*Z* genotype, and 9% of 64 females had the Z*W genotype. Further, ZZ* males had paler breeding plumage than homozygous ZZ males, and this genetic polymorphism is correlated with phenotypic differences (Schroeder *et al.*, 2008a).

Here, we present an analysis of correlations between length variation in an intronic amplicon used for molecular sexing and fitness-related traits in Black-tailed Godwits. Because type I statistical errors can never be excluded, we repeated the analysis of Schroeder *et al.*, (2008a) on covariation of CHD1-Z with plumage traits with a larger sample size. We then test for covariation of CHD1-Z with the fitness related variables of presumed quality of the breeding site, body mass, condition, correlates of reproductive success (egg volume and laying date) and adult survival. We additionally test for the occurrence of this variation in archived DNA from museum specimen from the beginning of the 20th century and discuss possible explanations of the observed patterns.

Materials and Methods

FROM 2004 TO 2007, WE CAPTURED 121 ADULT male and 163 female godwits on their nests in southwest Friesland, The Netherlands. Of these birds 203 came from our core-study area, the Workumerwaard (52°59'N, 5°24'E), which is described in detail by Schroeder *et al.*, (2008a) and by van den Brink *et al.*, (2008). The other 81 individuals were caught on surrounding farmlands and in nature reserves. Overall, 251 individuals (109 males and 142 females) were from nature reserves with restricted agricultural management schemes and 33 (12 males and 21 females) from intensively managed agricultural land. Birds were captured at the end of incubation (Schroeder *et al.*, 2008a), were weighed to the nearest g, and tarsus + toe length (± 1 mm) was measured. Each individual bird received an individual combination of four color rings plus a flag on their tibia.

To quantify plumage, digital pictures were taken of each captured bird with a resolution of 2272x1704 pixels using Nikon Cool Pix 4500 digital cameras. Seven plumage variables were scored by visual inspection of the pictures: (1) Bars score describes the extent of black bars on the belly on a scale from one to five. (2) Orange score is the intensity of orange a bird displays on the breast. (3) White in the head is the percentage of white feathers covering the head in side profile, with an accuracy of five percent. (4) White spots score is the percentage of the neck covered with white feathers, with an accuracy of ten percent. (5) Black spots score is the percentage of the neck covered with black spots with ten percent accuracy. (6) Back score is the extent of breeding feathers covering the back of a bird, on a scale from one to five. (7) The absolute number of breeding feathers on the back of a bird. For a more detailed description of these scores and their repeatability see Schroeder *et al.*, (2008a).

Length and width of all eggs in the nests were measured (± 1 mm), and egg volume was calculated by the formula $0.52 * \text{length} * \text{width}^2$ (Romanoff & Romanoff, 1949). Black-tailed Godwits have an invariant clutch size of four eggs (Cramp & Simmons, 1983). Hence, if a female decides for a high investment in a clutch she has to increase the volume of the eggs. The chicks are precocial and for the first few days of their lives they rely on energy stores left at hatching, which also affects chick survival during the first weeks after hatching (Bolton, 1991; Blomqvist *et al.*, 1997; Schekkerman *et al.*, 2008; our own data). Once godwit chicks fledge, annual survival is relatively high (0.70, unpublished data). Therefore, we consider egg volume as an indication of chick survival and therefore reproductive success. We do not have a more direct measure of reproductive success, because individual fledging success can only be determined reliably with radio transmitters in Black-tailed Godwit (see Roodbergen & Klok, 2008; Schekkerman *et al.*, 2008). Recruiting individuals were too few to be used in a statistical analysis. The start of incubation was estimated by measuring the degree of buoyancy of the eggs in water, as this is related to incubation stage (van Paassen *et al.*, 1984; Liebezeit *et al.*, 2007).

A blood sample of 20 μl was drawn from each bird from the brachial vein with a sterilized microcapillary tube. The sample was stored in 96% ethanol at -20°C for the

first four to six weeks and at -80°C thereafter. DNA was extracted using the chelex extraction method of Walsh *et al.*, (1991). Birds were sexed following the amplification protocol of Griffiths *et al.*, (1998). Fluorescently labeled PCR products were separated on an ABI 377 automatic sequencer and subsequently their exact length was determined using Genescan 3.1 software (Schroeder *et al.*, 2008a).

We collected small ($\approx 1\text{mm}^3$) skin samples from toe-pads of museum skins of 34 godwits from the years 1901–1931 from the Zoological Museum in Copenhagen. The skins were all collected at sites in Denmark. DNA from the skin samples was extracted with DNeasy TISSUE Kits from QIAGEN following the manufacturers' protocol in an archive-DNA clean laboratory at the Royal Ontario Museum (see e.g. Baker *et al.*, 2005). Birds were sexed with the primers M5 (Bantock *et al.*, 2008) and P8 (Griffiths *et al.*, 1998), which prime for a shorter amplicon of the intron than the combination P2 and P8 (Bantock *et al.*, 2008). The benefit of this method is that it has a higher success chance in partially degraded museum DNA. More importantly, it was shown to contain the same genetic polymorphism of the CHD1-Z in moorhens (Bantock *et al.*, 2008). We ran negative controls in both the DNA extraction and PCR to exclude artifacts. To verify that the genetic polymorphism observed with this new primer is the same as the one measured with the method of Griffiths *et al.*, (1998), we additionally genotyped seven female (two with the Z^* allele) and six male (three of them with the Z^* allele) contemporary DNA samples with known genotypes as controls with this method.

For each contemporary bird, only data of one capture occasion was used to prevent pseudoreplication. Body mass variation can result from variation in size or variation in nutritional stores (van der Meer & Piersma, 1994), and to differentiate between these two possibilities we estimated size-corrected body mass (hereafter called 'condition'). Step-wise linear regression was carried out with body mass as dependent variable and tarsus-toe length as predictor variable and sex as fixed factor. We used the standardized residuals of this analysis as an index of condition ($F_{2,275} = 300.2$, $R^2 = 0.69$, $P < 0.001$). Data on all plumage traits (bars score, orange score, white head, white spots, black spots, back score, breeding feathers) were combined in a principal component analysis. We extracted only factors with eigenvalues > 1 . The first two principal components (PC1, PC2) explained 63% of the variation in plumage traits (PCA: $KMO = 0.74$, $\chi^2 = 631.96$, $P < 0.001$). Birds that scored high on PC1 had more breeding feathers on their back, were more orange and had a larger extent of black bars on their belly; they also had less white plumage in head and neck. Birds that scored high on PC2 had more black spots on their neck. Principal component scores were normally distributed. We found no significant effects of PC2 and therefore do not report on this component from here onwards.

To confirm the results from Schroeder *et al.*, (2008a), we first tested univariately for differences in the plumage traits separately for both sexes with nonparametric Mann-Whitney U tests. We then performed GLMs on PC1, body mass and condition. Sex and Z^* were modeled as explanatory factors, and the interaction between them was used to detect differences between the sexes. Birds carrying the more frequent Z allele were coded as 0, and birds with genotype including Z^* as 1. Females were

coded as 0 and males as 1. As plumage may fade over the course of the season and nutritional status may change over time, we included date of capture as a covariate in the models.

We tested whether average egg volume per nest and laying date differed between nests of which at least one parent had a Z^* allele and nests of which none of the parents had the Z^* allele. As the genotype of both partners may influence the reproductive parameters, for the control group of that analysis we only used nests for which the genotypes of both parents were known to not contain the Z^* allele. This considerably reduced sample size, and to account for all birds, we additionally performed analysis on individual birds (ignoring the genotype of the partner) to determine whether average egg volume per nest and laying date differed between the sexes and genotypes. A GLM was performed with male and female genotype as explanatory factors (ZZ or ZW was coded as 0, ZZ^* or Z^*W as 1). For two males of the ZZ^* genotype, we had only data on one variable of reproductive output, which explains differences in sample sizes. Egg volume may decline over the course of the season, and egg volume and laying date also may vary between years (unpublished data). Therefore, laying date was modeled as a covariate with egg volume, and year as a fixed factor in both models. Laying date, season and year were not significant in any model and we therefore do not report statistics for these variables.

To determine the likelihood of missing a homozygous male (Z^*Z^*) in a sample the size of our data set we used a simple randomization model. Genotypes for 121 male birds (respective 92 for the core study area only) were drawn with the expected frequencies for being homozygous Z^* or not, and iterated 1000 times.

For the survival analysis, we assembled resighting histories of 190 individuals ringed as adults on the breeding grounds between 2004-2008. Individuals were recorded as being alive if caught or observed at least twice during the breeding period from February until July. Model notation follows Lebreton *et al.*, (1992). We first set up an a-priori global model with the parameters that were deemed important (sex, time). Goodness of fit (GOF) of this global model was tested with bootstrap procedures. We calculated the variance inflation factor by dividing the model deviance by the bootstrapped deviance. The model fitted the data well ($P = 0.20$). We used AIC to select the most parsimonious model (Akaike, 1973). As there was no evidence for strong overdispersion ($c\text{-hat} = 1.08$), we adjusted AIC values to allow for the extent of overdispersion measured by $c\text{-hat}$, using quasi-likelihood (QAIC). Preference for one model over another was based on $\Delta QAIC$ larger than two (Burnham & Anderson, 2002). To test for the effect of genotype on annual apparent survival (ϕ), we changed the most parsimonious model and made survival probability dependent on genotype and genotype * sex and report the change in $\Delta QAIC$.

We used R.2.7.1 statistical software (R Development Core Team, 2008) to compute statistics. We used the `lm()` function for constructing models and the `stepAIC()` function (both base package) to select the most parsimonious model by AIC (Akaike, 1973; Burnham & Anderson, 2002). We report parameter estimates $\pm SE$ for all effects that remained significant in the most parsimonious model, with covariates for correction

(year, date of season, laying date) included in the model, and F-statistics for each presented parameter and the final model. For the survival analysis, we used the program MARK (White & Burnham, 1992).

Results

eighteen (9%) Black-tailed Godwits carried the Z^* allele. We found no homozygous males with the Z^*Z^* genotype. No deviation from Hardy-Weinberg Equilibrium was detected in the dataset ($\chi^2_{\text{males}} = 0.78$, $P_{\text{males}} = 0.93$, $\chi^2_{\text{females}} = 0.38$, $P_{\text{females}} = 0.95$). Given the frequency of the Z^* allele in the population (8% of Z -alleles were Z^*), we expected 0.7% of all males being of the Z^*Z^* genotype, which of our 121 genotyped males would be less than one individual. The chance to miss a homozygous male by chance in a dataset of this size is 0.38.

We had data on reproductive success at 37 nests where both adult birds were of the more frequent ZZ or ZW genotypes, and of 38 nests where one bird was ZZ^* or Z^*W . No nest was incubated by two birds with the Z^* allele. All adult birds with the Z^* allele were caught breeding in nature reserve areas (33 out of 251), whereas none of them were caught on intensively managed agricultural land (33 birds). This difference is statistically significant ($P=0.02$, Fisher's Exact Test).

In general ZZ^* males had a paler breeding plumage compared with ZZ males. ZZ^* males had significantly fewer black bars on the breast and more white in the neck plumage than ZZ males, consistent with our earlier results (Table 8.1). There was no such effect of the Z^* allele in female godwits (Table 8.1). The first principal component (PC1) of male plumage traits differed between ZZ^* and ZZ males, the latter being more ornamented (Fig. 8.1). The interaction of sex*genotype was removed from the final model (Table 8.2, parameter estimate when in model:

Table 8.1: Univariate analyses of the effect of genotypic variation (ZZ , ZZ^* , ZW , Z^*W) on breeding plumage in male and female black-tailed godwits breeding in The Netherlands.

| | males | | | females | | |
|------------|--------------|-------|-------|--------------|-------|------|
| | N ZZ/NZZ^* | Z | P | N ZW/NZ^*W | Z | P |
| bars | 81/14 | -2.49 | 0.01 | 111/14 | -0.26 | 0.79 |
| orange | 85/15 | -0.06 | 0.96 | 114/14 | -1.74 | 0.08 |
| white head | 85/15 | -0.30 | 0.77 | 115/14 | -0.01 | 0.99 |
| white | 85/15 | -2.91 | 0.004 | 116/14 | -0.08 | 0.94 |
| black | 85/15 | -1.33 | 0.18 | 116/14 | -0.80 | 0.42 |
| back | 82/15 | -0.45 | 0.65 | 113/14 | -0.28 | 0.78 |
| feathers | 71/14 | -1.04 | 0.30 | 109/11 | -0.43 | 0.67 |

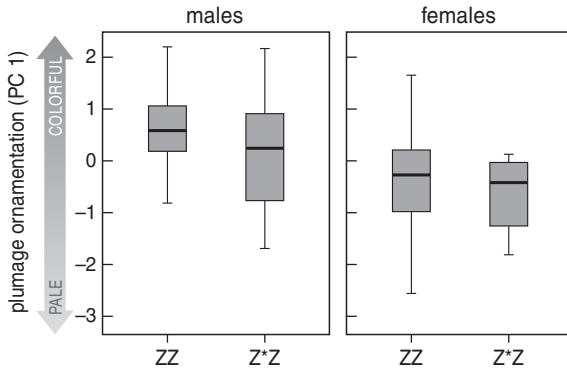


Figure 8.1: Phenotypic variation in plumage ornamentation of black-tailed godwit males and females with different genotypes on the CHD1-gene: ZZ, ZZ*, ZW and Z*W. Plumage ornamentation is presented as PC1 scores; birds scoring higher on PC1 are more ornamented than birds scoring low. Boxes depict the lowest and highest quartiles, lines through the Boxes indicate the median and whiskers the range of the observations.

$b \pm SE_{\text{sex} \times \text{genotype}} = -0.21 \pm 0.39$, $F_{1,187} = 0.28$, $P = 0.60$): thus although the effect seemed more prominent in males than in females (Fig. 8.1), we could not show this sexual difference statistically.

Z*W females were on average 13 g heavier than the more frequent ZW females (Fig. 8.2, Table 8.2). This was not the case in males; the interaction between sex*genotype remained in the most parsimonious model (Table 8.2). There was a trend for Z*W females to be heavier in relation to their size as evidenced by their higher condition (Fig. 8.2, Table 8.2), while we found no effect in males. Although the effect was not significant, the interaction of sex*genotype remained in the final model explaining condition (Table 8.2). We did not find a difference in body dimensions (tarsus+toe length) between the different genotypes. In a model, the interaction between sex and genotype, and genotype got removed from the final model and only sex remained (parameter estimates when in model: $\beta \pm SE_{\text{sex} \times \text{genotype}} = 0.41 \pm 1.82$, $F_{1,278} = 0.05$, $P = 0.82$; without interaction: $\beta \pm SE_{\text{genotype}} = 0.77 \pm 0.91$, $F_{1,279} = 0.14$, $P = 0.75$).

Nests with one Z* bird had a higher average egg volume compared with nests in which both of the incubating birds only had the Z allele ($t = -2.09$, $P = 0.04$; $N_Z = 31$, $N_{Z*} = 36$). This was mainly due to an effect of ZZ* males incubating at nests that contained larger eggs than those of other males ($t = -2.33$, $P = 0.03$; $N_Z = 103$, $N_{Z*} = 18$), whereas we did not find such an effect in Z*W females ($t = -0.44$, $P = 0.66$; $N_Z = 148$, $N_{Z*} = 15$, Fig. 8.3). A nest-independent GLM of individual genotype confirmed that eggs incubated by ZZ* males were 2 cm³ larger than eggs in incubated in nests by ZZ males, and female genotype did not remain in the most parsimonious model (Table 8.3). There was no effect of nests with at least one parent having the Z* allele on timing of breeding ($t = 0.75$; $P = 0.46$; $N_{ZZ} = 36$, $N_{ZZ*} = 31$). However, on the individual level, Z*W females initiated their clutches earlier ($t = 2.52$; $P = 0.02$; $N_{ZZ} = 148$,

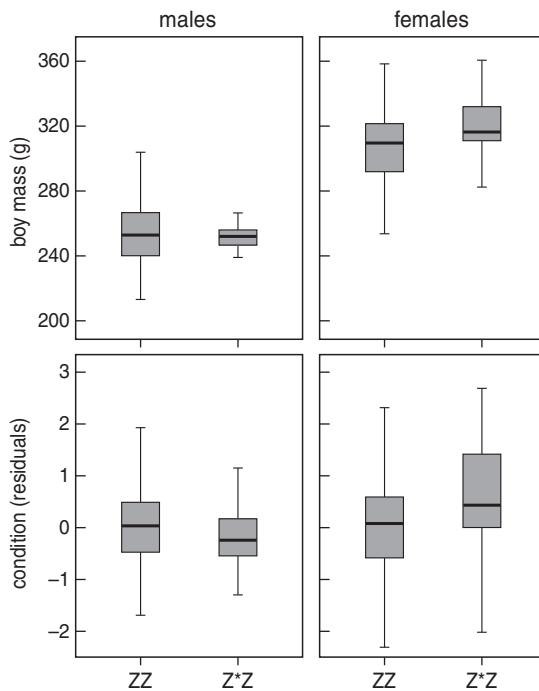


Figure 8.2: Body mass (g) and condition (residuals of a linear regression of body mass on tarsus-toe length, see text for statistics) of male and female black-tailed godwits in relation to genotypic variation on the CHD1-gene. Boxes depict the lowest and highest quartiles, lines through the Boxes indicate the median and whiskers extend to the range of the observations.

Table 8.2: Model results of the final GLM explaining black-tailed godwit breeding plumage ornamentation (measured as PC1), body mass and condition by genotypic variation on the CHD1-gene during late incubation. Date in the season was added to the most parsimonious model as a covariate. The F-statistics are for the final model including the (non-significant) date covariate (not presented). Coding: females = 0, males = 1; Z = 0; Z* = 1. PC1: $R^2 = 0.20$; $F_{3,188} = 17.27$; $P < 0.001$. Body mass: $R^2 = 0.62$; $F_{4,277} = 111.2$; $P < 0.001$. Condition: $R^2 = 0.03$; $F_{4,268} = 2.93$; $P = 0.02$.

| | $\beta \pm SE$ | F | p |
|-----------------------------|-------------------|--------|--------|
| plumage ornamentation (PC1) | | | |
| Genotype | -0.39 ± 0.20 | 3.81 | 0.05 |
| Sex | 0.91 ± 0.13 | 55.51 | <0.001 |
| body mass (g) | | | |
| Genotype | 11.64 ± 2.75 | 1.34 | 0.25 |
| Sex | -52.69 ± 2.75 | 440.53 | <0.001 |
| Genotype \times sex | -13.48 ± 8.07 | 2.80 | 0.09 |
| condition (residuals) | | | |
| Genotype | 0.43 ± 0.27 | 0.61 | 0.44 |
| Sex | -0.31 ± 0.13 | 9.03 | 0.003 |
| Genotype \times sex | -0.54 ± 0.38 | 2.06 | 0.15 |

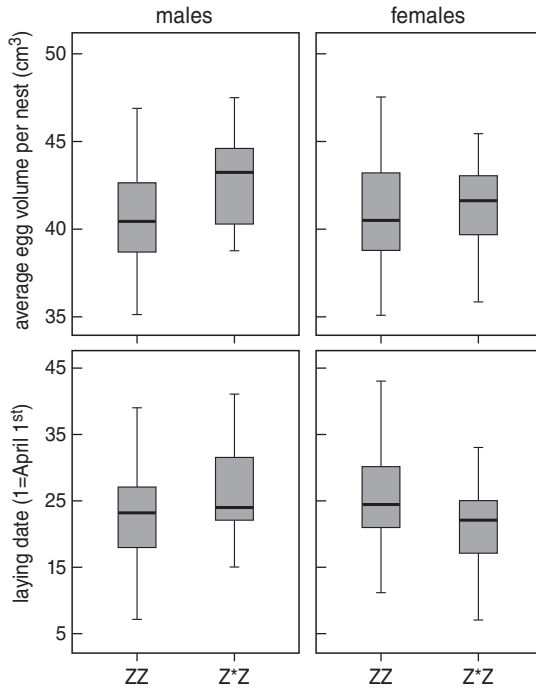


Figure 8.3: Average egg volume (cm³) and laying date in relation to the genotypic variation at the CHD1-gene of male and female black-tailed godwits. Boxes depict the lowest and highest quartiles, lines through the Boxes indicate the median and whiskers extend to the range of the observations.

Table 8.3: Results of the final model explaining black-tailed godwit average egg volume per nest and laying date as a function of genotypic variation on the CHD1-gene of the parents (whether or not a parent carries the Z* allele). Year was added to the most parsimonious model as fixed factor and, in the model with egg volume, laying date as covariate. The F-statistics are of the final model including (non-significant) year and laying date as main effects (not presented). Egg volume: $R^2 = 0.14$; $F_{5,67} = 2.26$; $P = 0.06$; Laying date: $R^2 = 0.11$; $F_{4,70} = 2.06$; $P = 0.09$.

| | $\beta \pm SE$ | F | p |
|--------------------|------------------|------|------|
| Average egg volume | | | |
| Male genotype | 1.68 ± 0.87 | 6.05 | 0.02 |
| Laying date | | | |
| Female genotype | -4.38 ± 2.15 | 4.27 | 0.04 |

$N_{ZZ^*} = 15$), but there was no effect of male genotype on timing of breeding ($t = -0.40$; $P = 0.69$; $N_{ZZ} = 103$, $N_{ZZ^*} = 18$, Fig. 8.3). The GLM on the individual genotypes confirmed that Z^*W females initiated their clutches on average four days earlier than ZW females, and male genotype was removed in the most parsimonious model (Table 8.3). Since in this model we did not distinguish between nature reserves and regular agricultural habitat, we repeated all above analyses (plumage, body mass, condition,

egg volume and laying date as response variables) on birds caught only in the core study area, the nature reserve with the highest sample size ($N_{ZZ} = 75$, $N_{ZZ*} = 17$, $N_{ZW} = 102$, $N_{Z*W} = 9$). These analyses gave qualitatively the same results as the full dataset, with lower significance values (all < 0.05). Similar results were obtained when using all nature reserves, including the core study area, but for laying date we detected no significant effect ($N_{ZZ} = 94$, $N_{ZZ*} = 18$, $N_{ZW} = 127$, $N_{Z*W} = 15$). This indicates that the links between genotype and fitness correlates do not arise due to a bias of the Z^* allele occurring only in nature reserves where fitness is higher (R. Kentie *et al.*, unpublished data).

In the most parsimonious survival model, adult survival was time and sex independent (Table 8.4). Resighting probability was high and independent of year ($0.90 \pm 0.02SE$). Annual adult survival estimated over the four years was relatively high ($\varphi = 0.95$). We found no support ($\Delta QAIC < 2$) for a statistical difference between this model and a model including genotype or a model including sex (Table 8.4, model 1 vs. model 2 vs. model 3). In the model that includes genotype, birds carrying the Z^* allele had statistically non-significant higher survival by 0.02 than birds with the more frequent allele (Table 8.5).

Table 8.4: Summary of model statistics of sex and genotypic variation on the CHD1-gene (Z^*) effects on adult survival of black-tailed Godwits breeding in The Netherlands.

| No. | Model | No. Par. | DQAIC | Q deviance | QAIC weight |
|-----|-----------------------------------|----------|-------|------------|-------------|
| 1 | $\Phi(.)P(.)$ | 2 | 0 | 70.45 | 0.38 |
| 2 | $\Phi(Z^*)P(.)$ | 3 | 1.75 | 70.17 | 0.16 |
| 3 | $\Phi(\text{sex})P(.)$ | 3 | 1.96 | 70.38 | 0.14 |
| 4 | $\Phi(.)P(t)$ | 5 | 2.24 | 66.57 | 0.13 |
| 5 | $\Phi(Z^*)P(t)$ | 6 | 3.82 | 66.10 | 0.15 |
| 6 | $\Phi(t)P(t)$ | 7 | 4.04 | 64.25 | 0.05 |
| 7 | $\Phi(\text{sex})P(t)$ | 6 | 4.18 | 66.46 | 0.05 |
| 8 | $\Phi(\text{sex} \times Z^*)P(.)$ | 5 | 5.41 | 69.75 | 0.07 |
| 9 | $\Phi(\text{sex} \times Z^*)P(t)$ | 8 | 7.45 | 65.58 | 0.01 |

Table 8.5: Survival estimates for black-tailed godwits breeding in The Netherlands for the three best supported survival models (Table 7.4).

| model | group | F | SE | 95%CI |
|-------|----------------------------|-------|-------|-------------|
| (1) | all adults | 0.950 | 0.019 | 0.894–0.976 |
| (2) | birds with Z^* allele | 0.968 | 0.034 | 0.778–0.996 |
| | birds without Z^* allele | 0.946 | 0.014 | 0.907–0.969 |
| (3) | males | 0.945 | 0.019 | 0.892–0.973 |
| | females | 0.952 | 0.019 | 0.894–0.976 |

In the DNA from the museum skin samples, the Z fragment of the M5-P8 method was 266 bp long, the Z* fragment was 262 bp and the W amplicon was 282 bp long and indicated the same length polymorphism than the P5-P8 primers. We successfully sexed 23 of the 34 museum samples (68% success rate). However, most likely due to PCR allelic dropout, the Z amplicon of three females could not be detected. We found the Z* allele to be present in one female (from the year 1929) among the remaining 20 samples of known genotype (59% success rate for determining genotype with respect to Z* allele). We found no correlation between genotyping success and age of the skin; successfully genotyped skins came from a range of years between 1901–1931.

Discussion

WE REPORT CORRELATIONS BETWEEN INTRONIC VARIATION on CHD1-Z and fitness correlates in male and female adult Black-tailed Godwits. This is the second species with a report on variation at this locus being linked with fitness-correlated traits (Lee *et al.*, 2002).

Earlier we showed that in Black-tailed Godwits, paler males pair with females that lay larger eggs, and are in better condition themselves (Schroeder *et al.*, 2009). Here we add that part of this effect may be associated with genetic variation of the Z-allele, too: Z* males are also paler and indeed paired to females producing larger eggs, and we detected a correlation with female body mass. Our estimate for annual survival is comparable to previous ones (Roodbergen *et al.*, 2008). There was a trend for birds of both sexes with the Z* allele to have a higher survival probability than birds with the more frequent allele. That this pattern was not statistically significant at the 5% level may be due to limited statistical power. As Black-tailed Godwits are long-lived, a slight increase in survival probability can mean a rather large increase in reproductive life. Moreover, lower survival in moorhen chicks with the Z* allele were reported by Lee *et al.*, (2002), suggesting that CHD-Z variation is linked to genes affecting survival in moorhens and possibly birds in general. It is therefore conceivable that such a correlation will eventually be shown to exist in Black-tailed Godwits. Despite the low frequency of the Z* allele, and consequently small sample sizes for the ZZ* and Z*W genotypes, the effect sizes were usually large, and the consistency of the patterns supports the notion that the correlation of genetic variation with fitness is real. All effects are in the same direction, lowering the chance that our conclusion is based on a type I error.

We found evidence that the genetic variation on CHDZ-1 was already present in the godwit population 80 years ago, indicating that the Z* allele is not a new mutation. This notion is supported by the fact that this mutation was found in a number of other bird species, which means that it is either old, or has arisen independently in many bird lineages. However, despite its apparent association with fitness, the frequency of the allele is relatively low. As the sample size of the historic data is small, we are unable to say whether the allele is changing in frequency.

We did not find assortative mating by birds with the Z^* allele. This is puzzling, given that Z^* females are of high quality and Z^* males are able to attract females of high quality, and thus assortative mating by genotype might be expected. We also did not find any homozygous Z^*Z^* males, which might be due to chance. It may also suggest that the fitness consequences of this variation are strongly asymmetric with respect to the different genotypes: a slight heterozygous advantage over the homozygous ZZ , but strong selection against the Z^*Z^* .

Associations between genetic and phenotypic variation mainly arises due to one of three reasons. (1) The polymorphism indeed affects phenotypic variation directly, (2) the polymorphism is linked (and in linkage disequilibrium) with other loci on the same chromosome which causally affect the phenotype or (3) the polymorphism reflects underlying, probably cryptic, population structure. (1) Since the observed CHD1-Z variation is expected to be neutral (located in a non-coding intron), we do not favour a direct causal relationship as an explanation. (2) However, the CHD1-Z locus may be physically linked with a gene(s) coding for or affecting the studied fitness correlates in godwits, resulting in the observed correlation between CHD1-Z variation and fitness. Even though genes influencing the expression of male plumage traits are most likely located on the Z sex chromosome (Sætre *et al.*, 2003; Gunnarsson *et al.*, 2007), it is unlikely that the CHD1-Z gene itself is responsible for this effect. This gene is known to have a role in transcription and gene expression, and therefore is expected to be very conservative and most likely not related to plumage (Stokes & Perry, 1995). It supposedly mediates chromatin structure and organization during transcription and is involved in interactions with DNA and RNA (Ellegren, 1996). Because all these are involved in basic protein synthesis CHD1 is considered a very conservative gene and should not have a fast mutation rate.

The genetic polymorphism may be linked to a different set of genes responsible for the fitness effects, by genetic linkage or epistasis (Lee *et al.*, 2002). Genetic linkage and epistasis occur more frequently when the linked alleles are on the same chromosome. This is even more likely if there is only one causal gene that affects a whole suite of traits including plumage ornamentation and body mass change, as recently suggest by Ducrest *et al.*, (2008). The differences between the sexes can also be explained by the fact that the Z^* polymorphism (including a linked causal gene) is on a sex chromosome. For example for body mass, the causal allele associated with Z^* is recessive, and therefore maybe only visible in females. Likewise, the causal allele for plumage might not be expressed in Z^*W females or suppressed by genes on the W chromosome. However, since data from families is, due to the low recruitment rates in godwits, not available, we can neither support nor exclude the possibilities that variation on CHD1-Z may directly or indirectly linked with genes affecting fitness.

The differential occurrence of the Z^* allele in breeding habitats of different quality indicates some degree of population structuring (3). Population structure is highly likely in Black-tailed Godwits as adult birds are highly faithful to their previous nest-site and in the relatively rare cases where they do change nest sites, dispersal distances are relatively short (Groen, 1993; van den Brink *et al.*, 2008; but see Schroeder *et al.*,

2008b). In a closely related subspecies, the Icelandic Black-tailed Godwit (*L. l. islandica*), it has been shown that nesting birds are partitioned by habitat quality: birds wintering on high quality foraging grounds are known to also breed in high quality breeding grounds and have a higher reproductive success (Gunnarsson *et al.*, 2005). In our case, this may mean that high quality birds – including those with the Z* allele – are more likely to be found on high quality breeding areas, and their offspring with the inherited Z* allele are likely to breed there, too. Using mitochondrial DNA control region sequences, Höglund *et al.*, (2009) did not detect any population structure in godwits breeding in The Netherlands. While it is currently not possible to distinguish between the three alternative explanations, we suggest that more extensive studies are required to detect cryptic population structure in the Dutch Black-tailed godwit population.

Acknowledgements

We want thank Jon Fjeldså and the Zoological Museum Copenhagen, who kindly allowed us to take toe-pad samples of godwits from their collection. We also thank It Fryske Gea, Fûgelwacht Warkum, Pedro Lourenço, Niko Groen, Petra de Goeij, Valentijn van den Brink and Freek Mandema for invaluable help in the field. Dick Visser provided help with the figures. This work was done under the license number DEC 4112B following the Dutch Animal Welfare Act Article 9. We were financially supported by a set-up grant to TP from the University of Groningen, a grant to JS from the Schure-Beijerinck-Popping foundation and a grant by the Vogelbescherming Nederland. Archive DNA analyses were supported by a National Science and Engineering Research grant to AJB. This work was done under the license number DEC 4112B following the Dutch Animal Welfare Act Article 9.

Nine

Seasonal carry-over effects: feather isotope signatures of diet during spring stopover correlate with female arrival at breeding site, body mass and egg volume in black-tailed godwits

Julia Schroeder, Pedro M Lourenço, Jos CEW Hooijmeijer, Christiaan Both, Marcel Klaassen, Francisco Santiago-Quesada, José A Masero, Juan M Sánchez-Guzmán & Theunis Piersma

Abstract

Over the last five decades black-tailed godwits (*Limosa limosa*) that breed in temperate grasslands have experienced great changes in habitat during their spring stopover en route from West-Africa to The Netherlands. They spend winter and spring staging mostly on rice fields where they only eat rice grains. Few birds also forage on natural fresh-water wetlands of marine mudflats. Different diets may lead to differential resource allocation during prealternate molt and migration, which in turn may affect reproduction. We used stable isotopes in feathers of black-tailed godwits from a breeding population in The Netherlands to detect a possible influence of diet on timing of arrival on the breeding grounds, on body mass during late incubation, and on correlates of reproductive success (egg volume and timing of breeding). Female godwits had lower δC , but not δN , signatures than males. Female arrival time, body mass but not size, and egg volume were positively correlated with δN , but not with δC . Although we cannot conclude the prey type, the quality of prey items on the spring staging areas affects reproductive success, both directly and indirectly.

Introduction

MANY MIGRATORY BIRDS spend most of their lifetime in places thousands of kilometers away from their natal and breeding sites, and the conditions on the staging sites may affect the fitness of individual birds (Cristol, 1995; Kokko, 1999; Bêty *et al.*, 2003; Bêty, Giroux & Gauthier, 2004). In detail, such carry-over effects are mediated through food abundance on staging areas (Ebbinge & Spaans, 1995; Marra *et al.*, 1998; Norris *et al.*, 2003). A first step towards understanding carry-over effects (Greenberg & Marra, 2004) incorporates analyses of food abundance and/or quality on the staging grounds and how these correlate with reproductive indices on the breeding grounds (Ebbinge & Spaans, 1995; Marra *et al.*, 1998; Norris *et al.*, 2003).

Black-tailed godwits (*Limosa limosa*), a migratory shorebird, provide an interesting case as over the last half-century many natural wetlands on the Iberian Peninsula used as spring staging areas were replaced by rice fields during the last century (Lains & Sousa, 1998; Kuijper *et al.*, 2006). Currently the main food during spring staging at the Iberian Peninsula consists of spilled rice (Sánchez-Guzmán *et al.*, 2007; Lourenço & Piersma, 2008a), while a smaller part of the populations utilizes natural fresh-water wetlands and mudflats (Kuijper *et al.*, 2006). Godwits of the nominate race (*L. l. limosa*) are currently suffering from a strong population decline at a rate approaching 30% over the last 15 years (Thorup, 2006; Gill *et al.*, 2007). A reduction in adult survival does not seem to be involved and the most common explanation for the decrease is low breeding success (Beintema *et al.*, 1995; Gill *et al.*, 2007; Roodbergen *et al.*, 2008; Hötter, Jeromin & Melter, 2007; Schekkerman *et al.*, 2008). Conditions on the spring staging grounds may contribute to these declines if different diets affect the timing of northward migration and breeding as well as body condition with cascading effects on reproductive success.

In the very closely related subspecies (Höglund *et al.*, 2009), the Icelandic black-tailed godwit (*L. l. islandica*), evidence for carry-over effects have actually been found. Individuals wintering in high quality saline areas arrive on the Icelandic high quality breeding grounds earlier than birds wintering in low quality freshwater areas (Gunnarsson *et al.*, 2006b). Further, birds that foraged during winter on more marine food were breeding in high quality areas and had a higher reproductive success than birds foraging on freshwater prey (Gunnarsson *et al.*, 2005b). Here we aim to explore the links between isotopic indices of spring staging diets and measures of breeding performance in individual black-tailed godwits breeding in the northern Netherlands. To do so, we study the relationships between stable carbon and nitrogen isotopic signatures of breeding feathers produced during spring staging in Iberia and the timing of arrival on the breeding grounds, body mass and size during late incubation as well as laying date and egg volume.

Methods

Field work

Data were collected from 2005–2007 in the Workumerwaard, Friesland, The Netherlands. For a detailed description of the study area see Schroeder *et al.* (2008a) and van den Brink *et al.* (2008). We visited godwit nests in the beginning of the incubation period and measured the length and width of eggs. A few days before the expected hatching date, we caught birds with walk-in traps on the nests. We weighed the birds to the nearest g and measured wing length (flattened and straightened, ± 1 mm), bill length (exposed culmen, ± 0.1 mm), total head length (± 1 mm), tarsus length (± 0.1 mm), and tarsus+toe length (tarsus plus mid-toe length without nail ± 1 mm). With scissors, we cut one breeding feather from the back of each adult bird. On the following days, nests were checked daily for hatching success.

A blood sample of 20 μ l was taken from the brachial wing vein and stored in 96% ethanol. DNA was extracted in the laboratory by the chelex extraction method (Walsh *et al.*, 1991). PCR products were acquired using the method of Griffiths *et al.*, (1998). Instead of an agarose gel, we separated the PCR products on an ABI 377 automatic sequencer and their length was determined with Genescan 3.1 software. Bird sexes were scored following Schroeder *et al.* (2008a).

We used average egg volume per nest as a component of reproductive success. Black-tailed godwits, like most waders, have an invariant clutch size and the correlation between egg volume and chick mass at hatching is very strong (own data). Egg volume is linked to hatchling mass, which in turn is linked to fledging success and thus egg volume can be used as a proxy for reproductive success (own data). Laying date and hatching date were calculated using the angle in which an egg flows in water (van Paassen *et al.*, 1984; Liebezeit *et al.*, 2007). Whether or not a nest successfully hatched was used as hatching success (binary variable).

Resightings of individually color-ringed birds have been made in January and February of 2005 and 2006 in Portugal and Spain on the main staging sites. The sites in Portugal, located alongside the Sado (38°24'N, 8°38'W) and Tejo (38°57'N, 8°54'W) estuaries, are rice fields. Godwits normally foraged on the rice fields and rested on the mudflats (a description of the areas can be found in Lourenço & Piersma, 2008a). From Portugal, it is known that godwits forage up to 94% on rice, and estimated 6% on animal prey items (Lourenço & Piersma, 2008a). The staging sites in inland Spain are approximately 300 kilometers west of the Portuguese sites, rice fields close to the village Hernán Cortéz in Extremadura, Spain (30°01'N, 5°55'W). There, rice fields represent the only suitable habitat for godwits and they are only seen there (for a description of the area we refer to Sánchez-Guzmán *et al.*, 2007). Here, godwits forage nearly exclusively on rice (J.A.M. & F.S.Q., unpublished data). We therefore expect the isotopic values to differ between the two sites with respect to trophic level and the closer proximity of estuarine prey in Portugal.

Arrival time on the breeding grounds was assessed by searching the breeding area for color-marked individuals from 1 March to 1 May 2005, 2006 and 2007, at least

three times per week. The daily resighting probability of individuals from the date of the first observation until the start of breeding was 0.37 ± 0.016 SE ($N = 2235$, based on 134 color-ringed individuals). The date a bird was first observed in a given year was used to indicate arrival time.

Stable isotopes

Stable isotopes are naturally occurring stable forms of elements, which differ in their nuclear masses. Stable isotopes are assimilated directly with the food and become fixed in the tissue during growth, for instance in a feather. Once made, feathers are metabolically inert. The stable isotope ratio remains; reflecting the composition of food at the time and place during which they were grown (Mizutani *et al.*, 1990; Hobson, 1999; Hobson & Clark, 1992; Rubenstein & Hobson, 2004). Since many migratory birds molt during spring migration, this offers a relatively low-effort method to get more information on the geographic origin of the birds (Hobson, 1999; Pain *et al.*, 2004; Rubenstein & Hobson, 2004; Yohannes *et al.*, 2006) and the possibility to link food quality on spring staging grounds to individual breeding success (Rubenstein *et al.*, 2002; Webster *et al.*, 2002; Norris *et al.*, 2003). We used two different isotopes: nitrogen and carbon.

We used breeding feathers from the back of Black-tailed godwits as sample material. These feathers are grown on the staging sites in January and February, when they molt into their breeding plumage (Cramp & Simmons, 1983; J.S. & P.M.L. pers. obs.). We additionally measured isotope ratios in prey items. Rice and invertebrate prey items have been collected in the study area (rice fields) in Portugal in 2006 and in the study area in Spain in 2007. Animal prey items were not sampled in the Spanish rice fields because densities were low.

Before further processing, the samples have been cleaned from surface contaminations using 96% ethanol and following this 99% hexane. Then, 200 μ g of the feathers were analyzed for carbon stable isotope ratio (parts per thousand, ‰, difference from the $^{13}\text{C}/^{12}\text{C}$ ratio in Vienna PeeDee limestone; further referred to as $\delta^{13}\text{C}$) and nitrogen stable isotope ratio (‰ difference from the $^{15}\text{N}/^{14}\text{N}$ ratio in atmospheric N_2 ; further referred to as $\delta^{15}\text{N}$) in a HEKAtech EuroEA elemental analyzer coupled on-line through a Finnigan con-flo interface to a Finnigan Delta S isotope ratio mass spectrometer. Reproducibility based on replicate measurements on a casein standard ($N = 145$) during the period of measurements was 0.12‰ (=SD) for both elements.

Statistical analyses

All body size measurements of black-tailed godwits (not including body mass) were collapsed in a principle component analysis. The first principal component (PC1) explained 77.72% of variation and is used in the following as variable “size”. Birds with a higher value of PC1 are larger than birds with a lower value. We used residuals of a linear regression of body mass on size as size-corrected body mass of the birds ($R^2 = 0.53$; $F_{1,78} = 86.99$; $P < 0.001$), further referred to as condition. We did not have size measurements of three birds and those were excluded from the analysis of size and condition.

Data on $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ were not normally distributed; with a log transformation of the absolute values we achieved normality. Body mass was standardized before statistical analysis. Average egg volume and laying dates were standardized per year to account for between-year variation. Repeatabilities were calculated following Lessells & Boag (1987).

We performed general linear models (GLMs) and included a factor for sex (females code as 0, males as 1) in all models. The godwits received individual color-rings on capture. Since we do not have sufficient data on first observation dates for the same season where we have data on stable isotopes we use the first observation dates in the subsequent year after catching for analysis, assuming that birds are repeatable in their diet on the staging grounds. This is a valid assumption since repeatabilities of isotopic signatures were quite high (see below). Because the carry-over effect may differ between the sexes, we tested for the interaction between sex and isotope signature. To account for between-year variation, we added a fixed factor coding for year to the most parsimonious models. Body mass, condition and egg volume might vary over the course of the season. To account for this we added laying date as a covariate to the most parsimonious models explaining these variables. Heavier females are known to lay larger eggs (own data). Therefore we added body mass as covariate to the most parsimonious models explaining variance in average egg volume. Since birds with a higher $\delta^{15}\text{N}$ have a higher body mass (this study), which in turn is expected to influence egg volume, we included the interaction between body mass and $\delta^{15}\text{N}$ to the model.

We used R.2.7.1 statistical software (R Development Core Team, 2008) and the `lm()` function of the base package for fitting linear models (Crawley, 2007). For fitting the logistic model we used the `glm()` function from the base package with the binomial link function. We used Akaike's information criterion to select the most parsimonious model employing the `stepAIC()` function from the base package (Akaike, 1973; Burnham & Anderson, 2002). We report ANOVA result tables for the models we tested, and parameter estimates with standard errors where all variables from the most parsimonious model and the correction factors year and laying date were in the model.

Results

WE COLLECTED DATA on stable isotope signatures of 40 male and 43 female black-tailed godwits breeding in The Netherlands in the years 2005-2007. Four female godwits were sampled in two years and between-year repeatability of the isotope values was high in $\delta^{15}\text{N}$ (0.56 ± 0.37 SE; ANOVA: $F_{3,7} = 4.73$; $P = 0.08$) and very high in $\delta^{13}\text{C}$ (0.86 ± 0.14 SE; ANOVA: $F_{3,7} = 13.08$; $P = 0.02$).

Male godwits breeding in The Netherlands had on average higher $\delta^{13}\text{C}$ values than females (Fig. 9.1A, males: -20.02 ± 0.68 SE; females: -22.55 ± 0.44 SE; $t = -3.12$, $P = 0.003$, $N = 83$). There was no difference in $\delta^{15}\text{N}$ values between the sexes (Fig. 9.1A, males: 10.59 ± 0.31 SE; females: 10.98 ± 0.33 SE; $t = 0.73$, $P = 0.47$, $N = 83$).

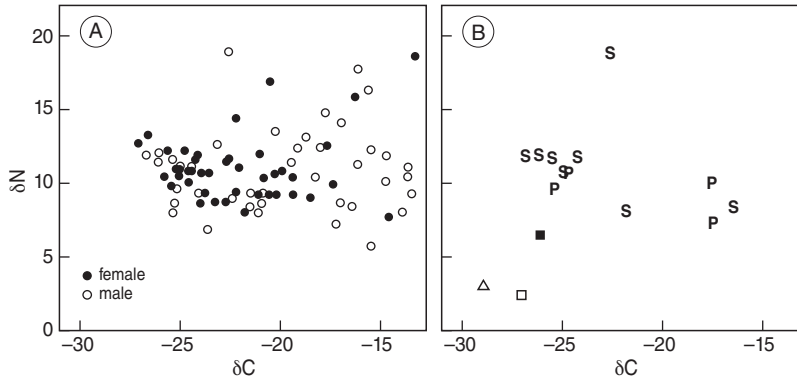


Figure 9.1: $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ measured in (A) breeding feathers of black-tailed godwits breeding in The Netherlands. Solid circles are female, open circle male godwits. (B) $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ measured in potential prey items in Iberia. The triangle depicts isotopic signature of animal prey items from Portugal, open squares depicts rice grains from Portugal, the filled square rice grains from Extremadura, Spain. Isotope measurements of breeding feathers of godwits resighted in the region are printed, S=resighted in Spain, P=resighted in Portugal.

Rice sampled in Spain ($N = 2$) had higher $\delta^{15}\text{N}$ and a comparable $\delta^{13}\text{C}$ value than rice from Portugal ($N = 4$) (Fig. 9.1B, rice $\delta^{15}\text{N}$: Spain = 6.4 ± 0.3 SD; Portugal = 2.38 ± 0.70 SD; $\delta^{13}\text{C}$: Spain = -26.13 ± 0.19 SD; Portugal = -27.03 ± 0.64 SD). Portuguese animal prey items ($N = 2$) had isotope signatures similar to those of Portuguese rice (Fig. 9.1B; $\delta^{15}\text{N} = 3.07 \pm 1.16$; $\delta^{13}\text{C} = -28.94 \pm 0.24$ SD). We observed 12 individuals in Iberia during spring migration for which we had collected feather samples: eight in Spain and four in Portugal. Their stable isotope values did not differ between staging areas or sex (Fig. 9.1B; $\delta^{15}\text{N}$ mean Portugal = 9.37, mean Spain = 11.66; $\delta^{13}\text{C}$ mean Portugal = -23.55 , mean Spain = -21.12 ; Anova: $\delta^{13}\text{C}$: $F_{\text{sex}} = 0.01$, $P = 0.92$, $F_{\text{location}} = 1.04$, $P = 0.33$, $df = 2,9$; $\delta^{15}\text{N}$: $F_{\text{sex}} = 0.09$, $P = 0.77$, $F_{\text{location}} = 1.67$, $P = 0.23$, $df = 2,9$).

On the basis of first observation dates of 17 male and 16 female godwits for which data on stable isotopes were known, average first observation date of these birds occurred on $21 \text{ March} \pm 1.11 \text{ day SE}$, and it did not differ between the sexes or years (Factorial ANOVA: $F_{\text{sex}} = 1.00$, $P = 0.32$, $F_{\text{year}} = 2.23$, $P = 0.14$, $df = 2,30$). Females with the highest $\delta^{15}\text{N}$ arrived on average 20 days earlier on the breeding grounds than females with the lowest $\delta^{15}\text{N}$ (Fig. 9.2), while there was no correlation in males (Table 9.1). We found no statistical effect of $\delta^{13}\text{C}$; the interaction between $\log -\delta^{13}\text{C}$ and sex and $\log -\delta^{13}\text{C}$ did not remain in the final model (Table 9.1).

Birds with a higher $\delta^{15}\text{N}$ were heavier. This effect was stronger in females than in males, and the interaction of $\log -\delta^{15}\text{N}$ with sex remained in the most parsimonious model (Fig. 9.3A, Table 9.2). $\log -\delta^{13}\text{C}$ and the interaction of $\log -\delta^{13}\text{C}$ and sex were removed from the final model (Table 9.2). Size was not explained by any of the isotopic signatures. All effects were removed from the final model but sex ($F_{\text{sex}} = 184.34$, $P < 0.001$, $df = 1,78$; all other effects $P > 0.32$ before removal). In the most parsi-

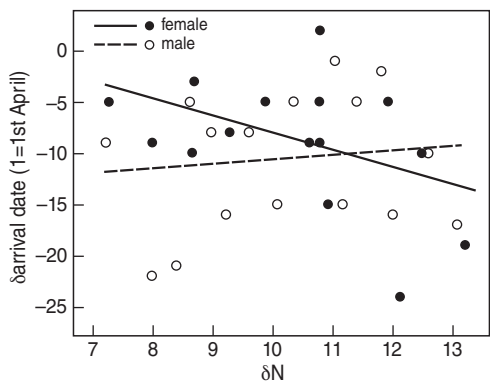


Figure 9.2: First observation date of female and male black-tailed godwits on their breeding grounds in The Netherlands in relation to the $\delta^{15}\text{N}$ signature of their back breeding feathers. Straight line is a regression line for females, dotted line for males. See Table 8.1 for statistics.

Table 9.1: Results of a GLM with first observation date (arrival) of Black-tailed godwits breeding in The Netherlands as dependent variable. AIC was used for the decision to reject effects.

| Variable | F _{4,28} | P | Estimate \pm SE |
|-----------------------|-------------------|------|--------------------|
| Sex | 1.57 | 0.33 | -63.43 \pm 29.25 |
| Year | 2.57 | 0.12 | -6.28 \pm 2.54 |
| Log(dN) | 1.87 | 0.18 | -25.14 \pm 9.84 |
| Log(dN) \times sex | 4.35 | 0.05 | 26.24 \pm 12.58 |
| Rejected effects | | | |
| Log(-dC) | 0.53 | 0.47 | -4.41 \pm 5.76 |
| Log(-dC) \times sex | 1.44 | 0.24 | 17.79 \pm 14.82 |

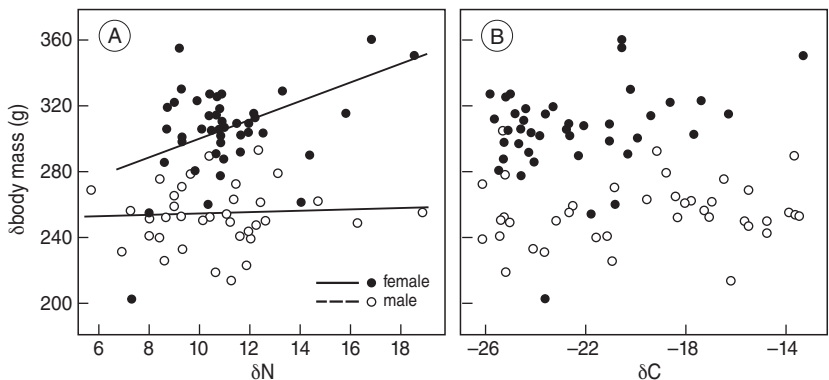


Figure 9.3: Body mass of female (bullets) and male (open circles) black-tailed godwits breeding in The Netherlands and in relation to (A) $\delta^{15}\text{N}$ and (B) $\delta^{13}\text{C}$ signature of their back breeding feathers. Straight line is for females, dotted line for males. For statistics see text and Table 8.2.

Table 9.2: Results of a GLM with body mass of Dutch Black-tailed godwits as dependent variable.

| Variable | F _{5,77} | P | Estimate ±SE |
|------------------|-------------------|--------|--------------|
| Sex | 44.11 | <0.001 | 2.79 ±1.69 |
| Year | 2.47 | 0.11 | -0.16 ±0.16 |
| Laying date | 0.01 | 0.92 | 0.002 ±0.009 |
| Log(dN) | 14.48 | <0.001 | 1.93 ±0.57 |
| Log(dN) × sex | 6.31 | 0.01 | -1.80 ±0.71 |
| Rejected effects | | | |
| Log(-dC) | 1.80 | 0.18 | -0.40 ±0.40 |
| Log(-dC) × sex | 0.10 | 0.75 | 0.28 ±0.91 |

Table 9.3: Results of a GLM with size-corrected body mass (condition) of Dutch Black-tailed godwits as dependent variable.

| Variable | F _{5,74} | P | Estimate ±SE |
|------------------|-------------------|------|--------------|
| SSex | 2.45 | 0.12 | 3.93 ±1.12 |
| Year | 1.52 | 0.22 | -0.13 ±0.24 |
| Laying date | 0.52 | 0.47 | 0.01 ±0.01 |
| Log(dN) | 2.22 | 0.14 | 1.97 ±0.92 |
| Log(dN) × sex | 2.62 | 0.11 | -1.81 ±1.12 |
| Rejected effects | | | |
| Log(-dC) | 0.96 | 0.33 | -0.47 ± 0.61 |
| Log(-dC) × sex | 0.001 | 0.97 | 0.04 ± 1.37 |

monious model explaining variance in condition, again the interaction between log $\delta^{15}\text{N}$ and sex remained in the model, but this effect was not statistically significant (Table 9.3). Log $-\delta^{13}\text{CC}$ and the interaction of log $-\delta^{13}\text{C}$ and sex were removed from the final model (Table 9.3).

Egg volume was higher in nests that were incubated by birds with a higher $\delta^{15}\text{N}$. This effect was on mainly due to a higher body mass in birds with a higher $\delta^{15}\text{N}$: the interaction of log $-\delta^{15}\text{N}$ and body mass remained (Table 9.4). There was a non-significant trend for nests that were incubated by birds with a higher $\delta^{13}\text{C}$ to contain larger eggs, independent of body mass (Table 9.4, Figure 9.4). We found no relationship between any of the isotopes and laying date, and all effects were removed from the most parsimonious model (all effects $P > 0.30$ before removal). Hatching success was not related to isotope value, and no variable remained in the most parsimonious model (binomial logistic model: $N = 89$, log $-\delta^{15}\text{N}$: Wald $Z = -0.48$, $P = 0.63$; log $-\delta^{13}\text{C}$: Wald $Z = -0.13$, $P = 0.89$).

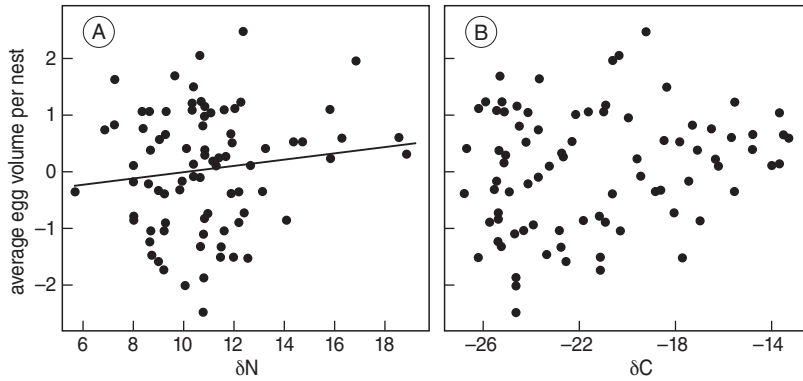


Figure 9.4: Average egg volume per nest standardized per year of black-tailed godwits breeding in The Netherlands in relation to (A) $\delta^{15}\text{N}$ and (B) $\delta^{13}\text{C}$ signature of their back breeding feathers. For statistics see text and Table 8.4

Table 9.4: Results of a GLM with standardized egg volume of Dutch Black-tailed godwits as dependent variable.

| Variable | $F_{5,77}$ | P | Estimate \pm SE |
|---|------------|------|-------------------|
| Year | 1.23 | 0.27 | -0.27 ± 0.24 |
| Laying date | 0.18 | 0.67 | -0.01 ± 0.01 |
| Body mass | 3.20 | 0.07 | -2.73 ± 1.08 |
| $\text{Log}(\delta^{15}\text{N})$ | 0.52 | 0.47 | 0.73 ± 0.57 |
| $\text{Log}(\delta^{15}\text{N}) \times \text{body mass}$ | 5.55 | 0.02 | 1.06 ± 0.45 |
| Rejected effects | | | |
| $\text{Log}(-\delta^{13}\text{C})$ | 2.29 | 0.13 | -0.75 ± 0.60 |
| sex | 0.19 | 0.66 | 0.27 ± 0.36 |
| $\text{Log}(-\delta^{13}\text{C}) \times \text{sex}$ | 1.20 | 0.28 | -1.10 ± 0.28 |
| $\text{Log}(\delta^{15}\text{N}) \times \text{sex}$ | 1.08 | 0.30 | 1.67 ± 1.68 |
| $\text{Log}(\delta^{15}\text{N}) \times \text{sex} \times \text{body mass}$ | 0.01 | 0.92 | -0.19 ± 1.90 |

Discussion

WHATEVER DIET COMPONENT yielded high $\delta^{15}\text{N}$ feathers correlated with female arrival, body mass during incubation and egg volume. What could these high $\delta^{15}\text{N}$ values represent? Nitrogen isotopes undergo trophic level discrimination and can therefore be used as dietary markers indicating the relative trophic level of the food the animal ingested (Thompson, Furness & Lewis, 1995; Kelly, 2000). However, the difference in nitrogen isotopic signatures between rice and animal prey items collected in Portugal was considerably smaller than the difference between rice collected in

Extremadura and in Portugal, preventing us from making strong statements about the trophic level of the food ingested (Fig 9.1B). That rice from Spanish staging areas had a higher $\delta^{15}\text{N}$ value than rice from Portuguese staging grounds might be due to a differential use of fertilizers: plants growing on soil fertilized with organic manure have a considerably higher $\delta^{15}\text{N}$ signature than plants growing on soil fertilized with artificial fertilizers (Denton *et al.*, 2001). Visual inspection of the isotope ratios of individuals resighted only in Spain indeed clustered towards higher $\delta^{15}\text{N}$ compared with birds only resighted in Portugal (Figure 9.1B). However, given the dual effects of degree of animal (estuarine) matter and nitrogen fertilizer on $\delta^{15}\text{N}$ signatures, we cannot distinguish godwits foraging on rice grains and animal prey items.

Isotopic signatures of $\delta^{13}\text{C}$ can indicate the degree of freshwater and marine habitat use with more negative values indicating a higher ratio of freshwater food and higher values suggest higher proportions of estuarine food items (Klaassen *et al.*, 2001; Gunnarsson *et al.*, 2005b). Surprisingly, however, the Portuguese food samples from estuarine areas had somewhat lower $\delta^{13}\text{C}$ values than the Spanish ones (Fig. 9.1B) although the differences were not statistically significant. As it is likely that Portuguese rice fields were irrigated with upstream river water, we suggest that the high $\delta^{13}\text{C}$ of the Portuguese birds might stem from marine prey items they may have ingested on nearby estuarine mudflats. The highest $\delta^{13}\text{C}$ signature comes from a Spanish bird, which suggests that this bird might have foraged on marine prey somewhere at the coast (e.g. in Portugal) before it was resighted in inland Extremadura. Our resighting data and carbon stable isotopes suggests that at least some godwits do forage on a variety of staging sites and those are most likely on a mixed diet that also includes marine prey. It is not clear yet where they ingest this marine prey, and next to the Portuguese coast it is likely that birds make use of other sites, like the Doñana Wetlands. This is an important staging site, but we did not have resightings nor prey samples from there (Rendon *et al.*, 2008). Overall, we do not find evidence for spatial segregation of staging godwits.

We showed that signatures for $\delta^{13}\text{C}$ differ between sexes. Females forage on average on food items with lower $\delta^{13}\text{C}$ values than males (Fig. 9.1A). This could indicate that females forage mainly on rice fields, while part of the males forage supplementary on prey with higher carbon isotope signatures, most likely marine prey (Fig 9.1A). Black-tailed godwits caught on Spanish rice fields did also show a similar difference between sexes in $\delta^{13}\text{C}$ measured of toenails and blood (JAM & FSQ, unpublished data). Because in that case, both sexes were caught on the same place, a spatial separation of genders is less likely. It may be that the timing of molt differs between the sexes, which could lead to such a divergence, but we have no evidence to prove this. This issue is clearly not solved yet and to do so we need more information on gender based food intake and migration schedules.

Although we cannot disentangle habitat and food sources, we do find evidence for a seasonal carry-over effect: Female birds that consume prey items with a higher $\delta^{15}\text{N}$ during wintering and spring staging arrived earlier on the breeding grounds and were heavier, but not larger and laid larger eggs than females foraging on sites and prey items resulting in a lower $\delta^{15}\text{N}$ (Figure 9.2A). These effects may be linked with each other: if

a female manages to be early on the breeding ground she has more time to gather nutrients needed to produce eggs, and can be fatter during incubation. Females heavier for their size were found to lay larger eggs, which could explain the correlation between the nitrogen isotope signatures and egg volume (Schroeder *et al.*, 2009). We did not find an effect of isotope signature on timing of arrival in males. This is surprising because theory suggests that early arriving males have an advantage in securing high quality territories, which in turn is expected to positively influence female mate choice and ultimately fitness (Kokko, 1999, Gunnarsson *et al.*, 2006b). One possible explanation is that as the differentiation in carbon signatures suggests, males and females may molt at different times, resulting in isotopic signatures that reflect the nutritional situation of different times for each sex. If this would be true, it would also suggest that the nitrogen isotopic value of males says a different thing than that of females.

We did not find significant effects of the carbon isotope signatures on individual traits on the breeding grounds, which is surprising since Gunnarsson *et al.* (2005b) found that birds that wintered in more saline habitats that resulted in higher carbon isotopic values breed in better quality areas and have higher reproductive output than birds wintering in inland areas that result in lower carbon isotope values. This study was based on averages of different godwit breeding areas, while we study individual traits (Gunnarsson *et al.*, 2005b). We did find a trend for birds with higher carbon signatures to incubate larger eggs, and it may be that this pattern would become more pronounced if we were to study it on the level of breeding areas differing in quality.

In conclusion, although we do not find evidence for spatial segregation, we do find support for the idea that female and male godwits differ in their prey or location choice on the spring staging grounds. Further, the choice of location and prey affects reproductive correlates on the breeding grounds, mostly in females. Since our results are mere correlations we cannot infer any causal relations and direction, - it may be that females of higher quality forage on higher quality prey items because they are of higher quality or that foraging on high quality prey makes a female better.

We suggest that data on resightings of color ringed individuals on other possible staging areas (Cota Doñana, Carmargue, Cádiz Salinas), together with an analysis of food items of these sites, could give a better insight to how different habitats and diets during spring staging affect reproductive output in the nominate race of the black-tailed godwit. Since these carry-over effects affect population dynamics it is important to take them into account when thinking about how to stop the ongoing population decline of the Black-tailed godwit in The Netherlands.

Acknowledgements

We want to thank It Fryske Gea, Fûgelwacht Warkum, Niko Groen, Petra de Goeij, Valentijn van den Brink, Rosemarie Kentie, Harry Korthals and Freek Mandema for invaluable help in the field and the analysis of stable isotopes. Fieldwork in The Netherlands was done under the license number DEC 4112B following the Dutch Animal Welfare Act Article 9. This work was financially supported by a set-up grant to TP from the University of Groningen, a grant to JS from the Schure-Beijerinck-Popping foundation (SBP/JK/2007-19), a grant to PL from the Portuguese Science Foundation and by the Vogelbescherming Nederland.

Ten

Synthesis

Julia Schroeder

General

IN THIS THESIS, we discuss how within-population individual differences are affected by changing selection pressures due to large-scale land-use changes, changes we humans impose on the environment. Traditionally, when the environment of a species is changed through human activities, we report demographic parameters to assess the situation. This is important for the monitoring of population size and the assessment of the extinction risk. However, as unpromising such situations are, we can additionally utilize such changes to learn about the biology of the individuals of the affected species (Piersma, 2006). This approach has been taken by many studies that unravel effects of climate change on the avian annual schedules (Crick & Sparks, 1999; Both & Visser, 2005; Visser & Both, 2005; Both *et al.*, 2006), and effects of changes in habitat available for populations (Baker *et al.*, 2004). Alike, we can also utilize other anthropogenic changes to learn about behavioural ecology. Especially in species that changed their traditional, natural habitat for an anthropogenically modified one it might be interesting and instructive to discuss impacts of human interference with nature from a historical perspective, on both, the individual and the population level. Sometimes, this may allow us to learn about how animals can adapt to a changed environment with phenotypic flexibility with respect to individual characteristics and life-history traits, and how they cannot. We may be able to unravel sequences of inter-correlated individual fitness traits that can affect demographic parameters in ways that may otherwise be hard to predict. Ultimately, such studies should help us find ways to reconcile human and animal requirements and find solutions that enable coexistence.

We discuss and examine the response of organisms to changes in the environment. What exactly is adaptive? The basic idea of adaptation is the change due to natural selection that makes an individual better suited to its environment (Townsend *et al.*, 2006). But in our framework of species coping with a changing world, it may mean two things: (1) a change in gene frequency, that leads to a change in trait frequency that benefits the bearer of the now more frequent trait expression. In this case, individuals with a certain genetic make-up are performing better, gaining higher fitness and adding more offspring to future generations than individuals with a different, less fit genetic make up. (2) However, some traits may also be phenotypically plastic, evolved to change with a changing environment. This adaptive response is called reaction norm. For instance, birds may decide to breed earlier or later, because that benefits their fitness in certain years while not in others. In this case, phenotypes change but not the underlying genetic variation, because birds may be able to breed early one year and late the next. The degree of phenotypic plasticity can provide us information on the original environmental variation of the organisms' natural habitat; it in a certain way is a display of the evolutionary history of that species. Hence a phenotypically plastic response to a changing environment evolved to benefit the fitness of the organism within its natural environment. But, if the environment change is unpredictable and unnatural, such *adaptive* (in the natural environment) phenotypic

responses can be *maladaptive* (in an environment that differs strongly from the natural one). Since individual responses to a changing world not only affect individual fitness, but also population dynamics, a maladaptive individual response can have severe population consequences.

In this work we often fail to distinguish between phenotypic plasticity and evolved adaptations. To make this distinction, one has to determine the genetic basis and the heritability of the focal traits, which provide basic information on the scope of evolutionary change (Falconer & Mackay, 1996). Further, determining reaction norms requires cross-foster experiments to disentangle the fitness effects of the genotype from those of the environment (Falconer & Mackay, 1996). Both experiments require knowledge on fledged and recruited offspring, something our study species is not suited for, methodologically and, most importantly, because of the extremely low recruitment rate.

In this last Chapter, I want to summarize our findings and put them in perspective. The dominating theme of this thesis is how human-made habitat changes throughout the range of Black-tailed godwits during the last century may have led to changes in selection pressures that act on fitness correlates. For black-tailed godwits breeding in The Netherlands, advancing and intensifying agricultural schedules together with climate warming have led to increased penalties for late breeding in black-tailed godwits (Haverschmidt, 1963; Zwarts *et al.*, 2009). Simultaneously, conditions for adults during egg production may have improved (Bijlsma *et al.*, 2001; Zwarts *et al.*, 2009). The switch from natural wetlands to agricultural breeding habitats most likely affected competition for nesting territories, and this in turn may have affected the importance of the information content of male plumage ornamentation. The remodeling of natural wetlands, or even of dry land, into rice fields on the Iberian Peninsula, and therewith a change in diet on the spring staging grounds, may also have influenced godwit biology. In this thesis, we discuss possible consequences of these changes for godwit life-history traits, sexually selected characters and demographic parameters.

The nature of changes inflicted by human activities over a long time span makes it impossible to conduct experiments. Therefore, the patterns we observed are correlative and aimed to provide testable hypotheses. Many of the variables my coauthors and I collected data on are correlated with each other and that makes it very difficult, if not impossible, to prove causation. To improve this situation, we gathered more data for supportive, yet circumstantial evidence on a different subspecies (the Icelandic godwit, *Limosa l. islandica*), and on historical datasets (Chapters 5, 6, 8). We also tried to test hypotheses and expectations derived from examining data collected over the last several decades and other research programs (Chapters 3, 6, 8). In addition, we repeated some of our analysis on larger datasets (Box B, Chapters 3, 4 and 8). We think that this data is valuable and the conclusions we draw may be of help to understand the phenotypic adaptive potential of black-tailed godwits, and also other meadowbirds, with respect to directional changes that humans are imposing on their habitat.

Timing of breeding

Optimal timing of breeding

Lack (1950) first brought up the idea of the optimal timing of breeding. The time when birds raise the highest number of offspring was suggested to be that time of the season when food availability for chicks peak. Subsequent studies showed that this view was conceptually right, but too simplistic, because it appeared that most birds behaved suboptimal: the majority of birds bred too late, and only the very early breeding birds met the optimal timing of breeding (Drent & Daan, 1980). Now, what if the optimal timing of breeding also depends on individual features of the parents with respect to the environment?

It was proposed that the nutritional state of a female during the phase of egg laying was important for the timing of breeding because it directly affects via energetic constraints the number and size of the eggs a female is able to lay (Drent & Daan, 1980; Daan & Tinbergen, 1997; Drent, 2006). Timing and quantity of egg production is affected by the nutritional state of the egg-laying female (Bolton *et al.*, 1992; Ratcliffe *et al.*, 1998). Since the amount of resources animals can allocate is limited, resources used for reproduction cannot be allocated to maintenance and self-survival (Stearns, 1992). Thus, the amount of resources a female is willing and able to allocate to egg production is assumed to affect her future survival. Females are selected to maximize lifetime reproductive output, and not annual reproductive output. This major trade-off between current and future reproduction is the basis of all life-history theory; it affects how females decide when to lay eggs, their number and size and is supposed to be the reason why so many females seem to lay too late.

The life-history context

The way this trade-off is accounted for in a particular life-history depends on the ecology of the species. At the extremes, there are species that have only a single reproductive season (semelparity) and a majority (to which all bird species belong) that reproduce more than once in a lifetime (iteroparity). In the first case, a female will put all her resources in the first (and last) reproductive attempt, at the cost on her own survival (Cole, 1954). In such species only the fitness of the chicks should affect timing of breeding (Bennett & Owens, 2002). In cyclic and somewhat stochastic environments however, iteroparity will be more successful (i.e. Wilbur & Rudolf, 2006; Simons, 2009). The benefit of such a reproductive strategy is the spreading of risk. If reproduction during one year fails due to external circumstances (stochastic catastrophic events), the next years offer more chances of producing offspring (Cole, 1954; Orzack & Tuljapurkar, 2001). In such species clearly the importance of adult survival and future reproductive success increases with the life expectancy. Extremely long-lived organisms are expected to shun unexpected costs to their offspring or even refrain from a year's reproduction if external conditions are too detrimental (Stearns, 1992; Erikstad *et al.*, 1998; Bennett & Owens, 2002; Orzack & Tuljapurkar, 2001).

While there is no true semelparous bird species, there is considerable variation in

mortality and reproductive rate (Bennett & Owens, 2002). Some bird species, among those short-lived passerines, reproduce only in two years on average, while others, for instance albatrosses and parrots have a reproductive lifespan of on average more than 50 years (Carey & Judge, 2001). We expect that such differences in life history affect decisions on the optimal timing of breeding.

The costs of reproduction

Life-history theory reflects on the trade-off between future and current reproductive success and relies on the assumption that reproduction is costly in terms of parent survival. This major assumption is often made, but also subject to debate (i.e. Cam *et al.*, 2002; Harshman & Zera, 2007; Ricklefs & Cadena, 2007; Mace & Pelletier, 2007; Weladji *et al.*, 2008). In birds, egg production is physiologically costly to laying females, but also costly with respect to life history traits (see review by Williams, 2005). Nutrition limits timing of egg production, but also, and especially when nutritional quality is considered, egg and clutch sizes (Williams, 2005). Within species, it has been shown that egg production does affect survival and/or fecundity of females (Monaghan *et al.*, 1998; Nager *et al.*, 2001; Visser & Lessells, 2001). In this thesis we have used egg volume as indicator of the investment of a female into reproduction. Our line of reasoning is since godwits nearly always lay a clutch of four eggs, females can only vary investment to clutches by varying egg size and the timing of laying. We did not consider egg quality and content. Egg volume has previously been shown to reflect female nutritional status at laying (Amat *et al.*, 2001; Sanchez-Lafuente, 2004; Karell *et al.*, 2008) and female godwits relatively heavier for their size lay larger eggs (Chapter 3, Box B). We therefore assume that average egg volume per nest is costly for female godwits and thus can be used as an index of female reproductive investment (Bonato *et al.*, 2009).

Optimization of reproduction in a long-lived farmland bird

In species with different food sources for adults and chicks, the phenologies of the two types may be differentially affected by environmental changes. Adult godwits forage on earthworms and leatherjackets, and the availability of these is to a certain extent positively affected by warmer winters and increasing amounts of fertilizers applied by farmers (Beintema *et al.*, 1985; Beintema *et al.*, 1995; Edwards & Lofty, 1982; Jordan *et al.*, 2004; Timmerman *et al.*, 2006; Zwarts *et al.*, 2009). Chicks, on the other hand, rely on insects dwelling in the grass canopy, whose phenology is earlier in years with warmer springs, but whose abundance is reduced considerably by mowing (Beintema *et al.*, 1991; Schekkerman & Beintema, 2007). This means that advancing agricultural schedules together with climate warming should have led to a higher and better food availability early in the season for adults, but also earlier, but unpredictable reductions in food availability for chicks (Bijlsma *et al.*, 2001; Schekkerman & Beintema, 2007; Zwarts *et al.*, 2009).

In Chapter 3 we speculate that godwits may decide on how much to invest in reproduction and when to lay eggs based on cues that are informative on adult food

availability during egg production. But while conditions for adults during egg laying have improved since the 1950s, conditions for offspring have become worse: warmer springs, improved plant species for more hay production and optimized fertilization schedules allow farmers to mow grasslands earlier and multiple times in the season (Haverschmidt, 1963; Bijlsma *et al.*, 2001; Zwarts *et al.*, 2009). Earlier mowing is dangerous for godwit chicks and clutches, and even lowers survival of chicks that survived the blades, especially for those born late in the season (Scheekerman & Beintema, 2007). Beintema *et al.*, (1985) proposed that godwits did advance their laying date for approximately two weeks between 1911 and 1974, however, it was suggested that they did not do this in response to increased penalties for late clutches but rather because of improved conditions for females during laying. In a dataset found in Haverschmidt (1963), the first egg date in Friesland advances by approximately 10 days from 1929-1962, and the arrival date of godwits in Friesland advanced alike (Chapter 3). Such advancement theoretically should have led to a longer breeding season and thus may have also allowed more second or replacement clutches. However since mowing advanced during that time, too (Haverschmidt, 1963; Bijlsma *et al.*, 2001; Zwarts *et al.*, 2009), we suggest that the breeding season got cut off at the end of the season and that (late) second clutches may have been of little importance.

For the time after 1976, we find no evidence for any further advance in the timing of laying in godwits (Chapter 3). Crane fly abundance (Tipulids), the major food for godwit chicks, depends on weather during summer of the previous year (Pierce-Higgins *et al.*, 2009). Such a lag in response can lead to an even more delayed response in the higher trophic bird populations whose chicks rely on the insects for food. For instance, in golden plovers (*Pluvialis apricari*), whose chicks like many other temperate breeding shorebirds forage on adult crane flies, productivity was related to the summer temperatures the pre-previous year (Pierce-Higgins *et al.*, 2009). Such a delayed response is difficult to detect, and it might be worthwhile to examine the patterns of timing of breeding in godwits with respect to such a mechanism. However, as for now, the reason of why godwits did not further advance laying date since 1976 despite an improvement of the conditions for the egg laying females, need further scrutiny and should be a fertile area of future work. We need to examine abundance and availability patterns of chick prey in relation to climate in natural areas, and relate timing of breeding to those. One possibility would be to conduct experiments with delayed or advanced timing of breeding, where eggs of nests of different incubation stages are swapped; together with radio tagging of the chicks to precisely determine fledging success. To determine the influence of anthropogenetic interferences at different levels, the very same experiment could be conducted in habitats of different quality. To determine the influence of the evolutionary natural history of the species, it could also be conducted on the Icelandic godwits, again in habitats of different quality. Instead of advancing laying dates, godwits increased investment into early laid eggs and decreased investment to late clutches (Chapter 3). It is unlikely that the changed reproductive investment is an evolutionary response to selection against late clutches. The average lifespan of an adult godwit is about 11 years, and the oldest godwit in the

EURING database reached nearly 30 years (Staav & Fransson, 2008; Roodbergen *et al.*, 2008). Selection by mowing only occurred over a few godwit generations and this time span may have been too short for selection by mowing to take place.

Therefore, phenotypic plasticity, - a response that evolved to be adaptive in a natural environment, may be a better explanation for our findings, no advance in laying date, increased investment early in the season during years with warmer winters and decreased investment later in the season in years with warmer springs. Thus, there have been two major changes in godwit fitness landscape; the patterns we observe can be a response to either one, or both. First, godwits may respond to increased penalties for late breeders due to earlier mowing; and second, they can respond to improved conditions, like the higher food availability early in the season. If we want to explain our findings in the framework of changed selection pressures, we need two components: (1) we need a functional explanation of cues godwits can respond to, and (2) we need to explain how the response to the cue discussed in (1) might have evolved, i.e. benefited godwits in a historical setting.

With respect to increased investment to eggs laid early in the season, we found no reliable cues that could predict timing of mowing at the time of egg laying (1) (Chapter 3). Therefore, the increased investment early in the season is unlikely a reaction to increased penalties for late clutches. However, it could be a response to higher food availability early in the season (1). Females may decide how much to invest into eggs depending on nutritional state, which depends on food availability, which is positively correlated with winter temperatures. (2) This explanation is valid in a natural setting (the original habitat of godwits, with no synthetic fertilizers), where food availability during egg laying maybe originally constrained how much a female could invest to her clutch. This would also explain why females invest less into eggs laid later in the season during warmer springs, when the soil is dried out and food is hard to get by (Chapter 3). Given that food availability and abundance for adult birds early in the season is higher and more homogenous in the agricultural grasslands than in natural wetlands godwits originally adapted to (Haverschmidt, 1963; Bijlsma *et al.*, 2001; Zwarts *et al.*, 2009), one could propose that godwits reacted to this increase in food by advancing laying date until a nutritional state-independent threshold was reached, and then instead invested the surplus of resources to increase egg size (2). It might even be that both processes happened at the same time, but we cannot make inferences on this since the data available to us is restricted to the time period after the advance in laying date.

I suggest that while godwits apparently do not adjust reproductive investment to the needs of their chicks, they do so to the needs of the adult, to maximize fitness: a surplus of food early in the season may have enabled females to lay earlier, and also larger eggs, while warmer springs and lower water tables may have constrained females in warmer years to produce larger eggs later in the season.

Linking individual life history decisions and population dynamics

Theory predicts that such unpredictability of the annual fitness prospects can support the evolution of longevity (Orzack & Tuljapurkar, 2001). Long-lived birds are normally

expected to be more *prudent* in their current reproductive investment, because they still have a long future reproductive life (Drent & Daan, 1980). They are expected to put the costs of current adverse environmental circumstances on their offspring, and base their reproductive investment less on the needs of the offspring than on those of the adult at the time of egg laying (Erikstad *et al.*, 1998). In comparison with godwits, in short-lived passerines the food peak for chicks seems to be of utmost importance for decisions on timing of breeding and reproductive investment (Visser *et al.*, 1998; Both & Visser, 2001; Cresswell & McCleery, 2003). In long-lived species, during shortages, adults are expected to invest less, abandon a clutch or even refrain from laying at all (Erikstad *et al.*, 1998; Weimerskirch *et al.*, 2001). The same trade-off is balanced out differently according to the life-history of the species (Bennet & Owens, 2002).

Our results suggest that godwits behave like theory predicts for long-lived birds: investment to annual reproduction follows a rather fixed scheme with little response in egg volume (Chapter 3), but not laying date, to environmental variation (Ricklefs, 1977; Erikstad *et al.*, 1998). Supporting this, a large part of our godwit breeding population was found to skip a reproductive season during years when either drainage or dry spells led to dried out, hard to penetrate soils and access to food for adults was restricted (P. L., unpublished data; G. J. Gerritsen, unpublished data). Thus godwits apparently maximize lifetime fitness by maximizing adult survival, and not annual reproductive output. Further, godwits start their autumn migration back to the winter quarters ever earlier, which can be seen as an indication for fewer second clutches laid after failing the first (Zwarts *et al.*, 2009).

If annual fluctuations are stochastic, and if the future after a bad year is indeed expected to be better, this strategy works well for all individuals. If circumstances change directionally, this prudent strategy may become maladaptive for birds that trade off reproductive investment for survival, because this brighter future will never occur. If godwits do not adjust laying date in their agricultural habitat, because that reaction may have been adaptive in a natural environment, it will have even worse consequences for reproduction, both for individuals but also on a population level. The few heavier chicks early in the season seem to be insufficient to counteract the high mortality of late offspring, and the population size will decrease. Reproductive output is too low to compensate for mortality (Schekkerman, 2008), causing the severe population decline shown by the Dutch black-tailed godwits (Fig. 10.1, Bijnsma & Mulder 2001; Thorup, 2006).

What could be done to set these things right? From a very simplistic point of view, making sure that conditions for offspring during chick rearing are a match to the conditions for adults during settlement and egg laying would be beneficial. As early as 1963, François Haverschmidt noted on the subject of a breeding population that disappeared within a few years time: “is it possible that a minor change in the ground waterlevel was responsible for the [...] disappearance of the godwits?” (Haverschmidt, 1963). Kleijn *et al.* (2004) suggest that high ground water levels are attractive to godwits when they make their settlement decisions. In general, one would expect that

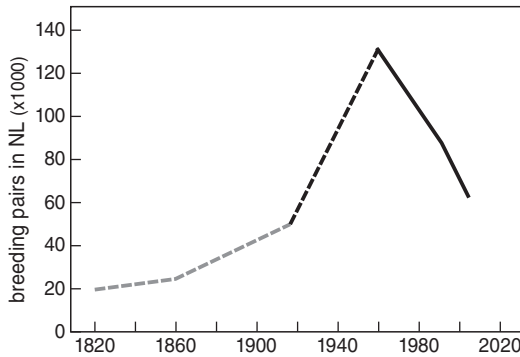


Figure 10.1: Population development of individual black-tailed godwits breeding in The Netherlands. Data pre-1960 are estimations based on data given in Bijlsma *et al.*, (2001). (Beintema *et al.*, 1995; Bijlsma *et al.*, 2001; Thorup 2006; Schekkerman, 2008)

high water levels also provide good conditions for insect food later in the season. Further, mowing should be postponed as long as possible, as advanced mowing dates not only increase chick mortality but also shorten the breeding season considerably (Schekkerman, 2008, Zwarts *et al.*, 2009). This is also not a new insight. I cite again François Haverschmidt: “Should grass drying [i.e. mechanical mowing early in the season, annotated by JS] be extended in the future, as its to be expected, it would appear that our meadow birds are doomed and the only solution would be to establish special reserves for them” (Haverschmidt, 1963).

Other factors like predation and/or the quality and conditions on wintering and staging grounds may play a role (Schekkerman *et al.*, 2009, Chapter 9). However, the most frequently named measures to improve the situation of godwits in The Netherlands, higher water tables throughout the breeding season, and late and cautious mowing, are all measures that also lead to a lower harvest. Thus it is not possible to reconcile economic expectations of farmers, who want to maximize grass-land yields, with the wish to increase godwit reproductive output.

Long-term and large-scale changes happened not only on the breeding grounds, but also on the staging sites on the Iberian Peninsula. In Chapter 9, we studied a possible relationship between the food ingested during spring staging and reproductive parameter on the breeding grounds.

Habitat changes on staging sites

FOR BLACK-TAILED GODWITS, the staging habitat and diet on the Iberian Peninsula did change dramatically during the last decades (Kuijper *et al.*, 2006). Godwits used to forage in natural wetlands, assumedly on a mixed diet of naturally occurring insect larvae, worms and plant seeds, plus marine prey items. Nowadays, the main spring

staging areas are agricultural rice fields in Spain and Portugal (Lourenço & Piersma, 2008b; Sánchez-Guzmán *et al.*, 2007). The abundance of animal prey items on these rice fields is low and fecal sample analyses from godwits staging there contained 94% of rice grains (Lourenço & Piersma, 2008a). Thus diet for godwits in Iberia probably changed from a well-balanced mixed diet to an apparently monotonous rice diet, and the implications of this change are not clear.

Our results from Chapter 9 suggest that a diet that results in a high $\delta^{13}\text{N}$ value would most likely be beneficial. Such a diet would be one that consists of animal prey items, and marine prey. However, our results indicate that a diet on rice grown on highly fertilized soil could also lead to such isotopic signatures and we thus were not able to make definite statements on the consequences of a shift towards a diet with a high rice percentage. We are currently collecting more prey samples from more locations to be analyzed.

We conducted experiments on the effect of a rice diet versus a diet on fly larvae on black-tailed godwit body mass and plumage development. We found that godwits feeding for six weeks on rice only did not gain much mass during that time, in contrast to birds feeding on fly larvae (*Box C*). This suggests that a diet on rice alone may be not sufficient for godwits to fatten up during spring staging. Since we also observed a higher level of aggression of birds that were fed rice only, we cannot conclude causality, or a direction of causality. We cannot be sure whether the energetic contents of rice lead to a lower body mass, or whether eating rice in captivity led to higher aggressiveness which in turn led to lower body mass. Calculations of energy budgets and digestive efficiency suggest that a pure rice diet (*ad libitum*) should be sufficient for a godwit to gain weight and fatten up for migration (P.M. Lourenço, pers. comm.). Therefore we still do not know what effect different diets during spring staging have on black-tailed godwits.

During spring staging, godwits start molting into breeding plumage (Zwarts *et al.*, 2009). Plumage ornamentation is often used for signaling individual quality during mate choice and reproduction, and is expected to covary with fitness correlates. In Chapters 4-7, we examined the sexually selected plumage ornamentation, and the effect of changing selection pressures on it, in black-tailed godwits.

Plumage ornamentation and the loss of sexual plumage dimorphism

BLACK-TAILED GODWITS ARE SEXUALLY DIMORPHIC with respect to plumage ornamentation and males are usually more colorful than females (Chapter 4). Theory of sexual selection predicts that sexual plumage dimorphism occurs when ornamentation is favored by selection more in one sex than in the other (Andersson, 1994). Surprisingly, Chapter 5 reports an opposing selection pressure: godwit males that were more colorful and more ornamented were smaller, lighter and paired with females that laid smaller eggs than paler males. And larger eggs produce larger chicks that are more likely to survive, - larger eggs mediate higher reproduction (Chapter 3).



Figure 10.2: Degree of sexual dimorphism of univariate breeding plumage scores of black-tailed godwits over time. Data from Chapter 5 was used. We calculated for each time category (1840–1899, 1900–1949, 1950–1999, 2000–2007) an index of dimorphism for the plumage scores that change over time (bars: straight line, orange: dashed line, back: dotted line; $(1 - \text{Score}_{\text{male}} / \text{Score}_{\text{female}})$). For white in head (dashed-dotted line) we used the reverse index. This is a measurement of coloration rather than paleness and easier to compare with the other scores. The higher this value of the indices of dimorphism, the more colorful males are with respect to females. If this value reaches zero, there is no difference between males and females.

Only few examples exist where males with a less exaggerated sexual trait gain higher reproductive success. Sætre *et al.*, (1997) found that pied flycatchers *Ficedula hypoleuca* breeding females prefer duller brown males over more colorful ones, in areas where they in sympatry with the collard flycatcher, *F. albicollis*, perhaps to avoid hybridization. In an island population of the house sparrow *Passer domesticus*, females preferred to mate with males that have smaller badges, although this was not found again on data of the same population but from later years (Griffith *et al.*, 1999, but see Nakagawa *et al.*, 2007, own data). In 2007, Delhey *et al.* found that blue tits (*Cyanistes caeruleus*) males with a lesser-exaggerated male trait experienced higher reproductive success. However, the underlying mechanism was apparently age: older males were less ornamented, but also more experienced and thus a correlative pattern with reproductive output arose (Delhey *et al.*, 2007). In black-tailed godwits, museum skins of male godwits from 100-150 years ago were more colorful than the ones today (Chapter 5). It is unlikely that this effect is due to storage time, since storage effects are usually small. Further, dust would have the opposite effect (older skins to be paler than younger ones). It has recently been suggested for melanin coloration that over time, the proportion of eumelanin may decrease, which could lead to an increase of overall reflectance (Doucet & Hill, 2009). However, if we were to find such an effect, we would expect to find it in both sexes alike, but we only find it in male skins. The orange-reddish and black plumage ornamentation of male godwits gradually faded over these 150 years, and we suggest that sexual dimorphism in plumage decreased (Fig. 10.2).

Phylogenetic studies have revealed that loss of elaborate male traits, that before were maintained by sexual selection, is widespread (Burns, 1998; Wiens, 2001). The

expression of an honest, sexually selected signal is a balance between natural selection pressures, male condition and female preferences (Andersson, 1994). Environmental changes of habitat can influence the strength of sexual selection and also the opposing, natural selection. Therefore, when environmental changes occur, it may be that the costs of an ornament become too high to be maintained. Then males that do not pay the costs but invest in reproduction would have an advantage over males that maintain ornamentation (Wiens, 2001). An often-cited example is found in fish. Differential predation pressure between populations of guppies (*Poecilia reticulata*) living in different streams lead to differential expression of male coloration (Endler, 1983). If predation pressure increases, it may be too costly for males to maintain conspicuous ornaments (Wiens, 2001).

For the godwits, this means that the very same selection pressures that once favored more ornamented males (something that seems to occur in a congener, the bar-tailed godwit (*Limosa lapponica*, Piersma *et al.*, 2001; Drent *et al.*, 2004) and a subspecies, the Icelandic godwit (*L. l. islandica*, Chapter 6)) must have changed, disappeared or at least, weakened. Since this happened over the last 150 years, changes in the environment that occurred during this time must be responsible for this decrease in sexual plumage dimorphism (Fig. 10.2). In search for an explanation, we review what changed for godwits on the breeding grounds during this time period.

Environmental changes on the breeding grounds as explanation

From early 1800 onwards, the bogs and swamps that were the original breeding habitat of black-tailed godwits in The Netherlands were lost and changed into agricultural areas (Bijlsma *et al.*, 2001; Zwarts *et al.*, 2009). At the end of the 19th century, when more and more farmers started using artificial fertilizers, black-tailed godwits left their natural breeding habitat to breed in agricultural grassland (Haverschmidt, 1963; Beintema *et al.*, 1995; Bijlsma *et al.*, 2001). Although not much data is available, we know that the population of godwits in The Netherlands increased considerably after the move (Fig. 10.1; Haverschmidt, 1963; Bijlsma *et al.*, 2001; Zwarts *et al.*, 2009). The higher quality of the grasslands in terms of food abundance for godwits triggered this move (Beintema *et al.*, 1995; Bijlsma *et al.*, 2001; Zwarts *et al.*, 2009), and afterwards, the population size increased considerably (Fig. 10.1; Beintema *et al.*, 1995). An increase in population size can only be due to an increase in one or both of the demographic parameters adult survival and reproductive output. An increase in reproductive output could have taken place due to the improved conditions on the breeding grounds. However, at the very same time, the large-scale creation of rice fields on the Iberian peninsula took place and it is unclear yet whether that affected adult winter survival positively or negatively. We can only speculate which was the case for godwits at that time (Figure 9.2).

However, from 1960 onwards, with ongoing advances in agricultural practice, the wet, extensively managed meadows made room for intensive agricultural fields (Beintema *et al.*, 1997; Bijlsma *et al.*, 2001). Since then, the population decreased by

nearly 50% (Thorup, 2006; Zwarts *et al.*, 2009). This was due to lowered reproductive output, not to lowered adult survival (Roodbergen *et al.*, 2008).

This also means that since recruits are scarce, the adult population grows older (Zwarts *et al.*, 2009). If older godwits are paler than younger adult godwits (personal observation N.M. Groen), the population average plumage coloration would decrease as the population age increases. In addition, if more experienced, older godwits gain higher reproductive output, this could explain the pattern we find. However, this explanation is based on the assumption that older godwits are paler than younger ones and there are no studies to support it. We made an attempt to test this for the univariate plumage scores in Chapter 4. We now have repeated plumage measurements on seven females and seven males, one of each sex of three years and six each sex of two years. We calculated repeatabilities of the first principal component (PC1) (Becker, 1984; Lessells & Boag, 1987). PC1 was highly repeatable in males (Figure 9.3, $R = 0.79 \pm 0.14$, $N_0 = 2.13$, $N = 7$, $F = 12.05$, $P = 0.001$) and less so in females ($R = 0.60 \pm 0.24$, $N_0 = 2.13$, $N = 7$, $F = 4.51$, $P = 0.02$). Repeatabilities of the univariate plumage scores are equally high (Chapter 4). Visual inspection of Figure 10.3 does not support the idea of plumage ornamentation changing considerably between consecutive years.

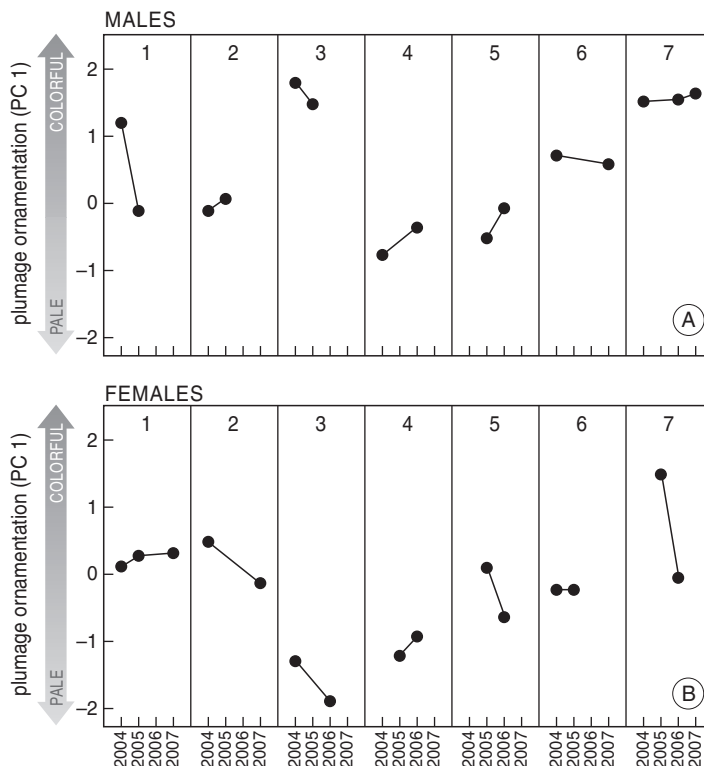


Figure 10.3: Changes in plumage ornamentation of individual male (A) and female (B) black-tailed godwits breeding in The Netherlands over four years.

To my current knowledge, no method exists on age determination in adult godwits, so we cannot refine this analysis. However, since godwits are long-lived (Staav & Fransson, 2008, Roodbergen *et al.*, 2008) and we only cover five years of data, we cannot exclude a possible age effect.

Another change in godwit breeding environment is increased predation pressure mainly by increasing populations of birds of prey, which was suggested to be one of the many factors that drive the population decline (Bijlsma *et al.*, 2001; Teunissen *et al.*, 2008; Schekkerman *et al.*, 2009). In godwits, both sexes incubate the eggs, which are laid in a nest on the ground (Beintema *et al.*, 1997). As the habitat structure changed during the last 150 years (Bijlsma *et al.*, 2001), it is likely that the detection probability of an incubating bird by a bird of prey changed, too (Schekkerman *et al.*, 2009). This might have led to higher detection rate of nests incubated by conspicuous birds, and females that choose for less bright males would have a reproductive advantage over others and might be more prone to invest more into reproduction, and lay larger eggs. Nonetheless, these two explanations (ageing population and increased predation pressure) are not very satisfying because they fail to explain why godwits already became paler during the first half of the twentieth century, when the population was growing and predation pressures were lower.

I propose another scenario. During the time of population expansion, the availability of high quality nest sites must have been high, and therefore, competition among males for territories must have been relatively low. It may well be that it was no longer important for a male to signal his abilities to gain a high quality territory with a colorful plumage, since every male, regardless of his qualities, must have been able to secure a good quality nesting site. Hence, being colorful may not have yielded the benefits of higher reproductive output any longer, and sexual plumage dimorphism decreased. Further, paler males may have been able to breed closer to conspecifics, which might have led to a stronger predation defense due to more individuals that would take part (*Box C*). This could have led to females investing more to clutches that are better defended. I will first explain why I suggest a change in competition for nesting sites and later go into detail on the benefits of being pale and the costs of being colorful.

The idea that competition for high quality nesting sites is connected with male godwit plumage coloration implies specific assumptions on what led to the sexual plumage dimorphism in the first place. In a natural setting when competition for nesting sites may have been high, and where selection pressures may have had the opposite effect, we would expect more ornamented male godwits to be more successful than pale godwits (Piersma & Jukema, 1993; Piersma *et al.*, 2001; Drent *et al.*, 2004). We collected more correlational data on populations that breed in a different environment to gather more circumstantial support.

We conducted a study on male plumage coloration in relation to egg volume on the Icelandic subspecies, *Limosa limosa islandica* (Chapter 6). The most striking difference between both subspecies is the current population trend: the *limosa* population is in strong decline, while the *islandica* population is increasing (Gill *et al.*, 2007).

Another difference is the breeding habitat: in The Netherlands, godwits breed on agricultural grassland, which is most often intensively managed (Beintema *et al.*, 1995). In Iceland, godwits breed on, from a Dutch point of view, less than extensively managed grassland and on near-natural dwarf-birch bogs (Gill *et al.*, 2007). However, the latter is considered low-quality habitat for godwits, while only the former, extensively managed grasslands are high quality areas with higher reproduction rates than the low quality areas (Gunnarsson *et al.*, 2005a). It was suggested that the high quality areas resemble the former low-intensity agricultural fields that godwits found so attractive in The Netherlands at the beginning of the 20th century (Gill *et al.*, 2007). In the contemporary situation, the nominate population is in strong decline. The most often named cause for this is a strong reduction of reproductive output, most likely due to high mortality of chicks after hatching. The pre-egg-laying situation however, is different. It is likely that the average breeding site a Dutch black-tailed godwit can secure is of higher quality – with respect to food for adults pre-egg-laying – than that of one in a natural setting.

In contrast with The Netherlands, such high quality breeding areas are rare in Iceland. The increase in population size in Iceland occurred concurrent with an increased colonization of low quality habitat, which is seen as an indication for the high quality areas to be filled up (Haverschmidt, 1963; Gunnarsson *et al.*, 2005b). Thus, in the Icelandic godwits, competition for nesting sites is supposedly strong and male plumage ornamentation may be indicative of male-male competition. A more colorful male might be able to secure a high quality-nesting site, and on such a site, a female may be more likely to invest much into reproduction. As expected, we found a positive correlation between male plumage ornamentation and egg size in Icelandic godwits (Chapter 6) – opposite to the pattern in the nominate species (Chapter 5). This supports the notion that competition for nest sites may be linked to male plumage ornamentation in black-tailed godwits of both subspecies, with selection acting opposite on both populations.

This explanation for the decrease of plumage sexual dimorphism in the nominate species has an important shortcoming. We find that paler males of the nominate species were in a better condition and mated with females laying larger eggs. However, if ample availability of high quality territories led to all males securing high quality territories regardless of their quality and ornamentation, then why should paler males have a fitness advantage over more colorful males? If signaling a colorful plumage is no longer important we would expect no fitness differences between males differing in ornamentation. Since paler males performed better, and the frequency of pale males increased since 1840, we expect selection for paler males or selection against colorful males. This means we have to be more specific and identify the costs of being colorful and the benefits of being pale, and discuss how those costs changed over time.

The costs of being colorful

The coloration of godwit breeding plumage is melanin-based (Toral *et al.*, 2008). According to the handicap principle, sexually selected signals must be costly to be reli-

able; otherwise they cannot function as honest indicators of a male's qualities (Zahavi, 1975; Pomiankowski, 1988). Until recently, it was unknown how the expression of melanin-based ornaments could invoke physiological costs (Griffith *et al.*, 2006). One idea to explain the costs of melanin plumage coloration was the existence of a pleiotropic link between steroid levels and melanin-based coloration (Almasi *et al.*, 2008; Ducrest *et al.*, 2008; Roulin *et al.*, 2008).

The gene responsible for the production of melanocortins, and therefore also melanin coloration, is the pro-opiomelanocortin gene (POMC). The main site of melanocortin synthesis is the pituitary gland, but also the melanocytes. There are four main receptors for melanocortin (MC1-5R), and only one of those (MC1-R) regulates melanin coloration while the other play roles in several physiological and behavioral functions, among those aggressiveness, stress response and steroid levels (Prota, 1992; Nadeau, Burke & Mundy, 2007; Ducrest *et al.*, 2008). Therefore, covariation between melanistic coloration and sexual behavior, steroid levels, aggressiveness, stress coping, immune response, metabolic rate, body mass and size is expected and observed in a range of species (McGraw *et al.*, 2003; for an overview see Ducrest *et al.*, 2008; Safran *et al.*, 2008).

The actual causal relationships between these traits – whether steroid levels affect plumage ornamentation and behavior; whether plumage ornamentation affects the behavior of conspecifics and in response that of the bearer, and then hormone levels or any other combination of causality chains – is not well understood, and apparently not unidirectional (Safran *et al.*, 2008, Rubenstein & Hauber, 2008). It was suggested to be a dynamic feedback system (Rubenstein & Hauber, 2008). In such a system, dynamic feedbacks between steroid or androgen levels, and linked behavioral costs may govern the evolution and maintenance of sexually selected plumage coloration (Rubenstein & Hauber, 2008). Genetic differences may lead to individual variation in melanin expression and or individual variation of susceptibility to hormone level changes. Further, between-individual variation of such a dynamic feedback system within single traits and their costs, may occur due to the environment, while the social environment in turn may be affected by the individual itself. For instance a colorfully ornamented bird that is exposed to other aggressive birds may react differently than a bird that is surrounded by relatively peaceful conspecifics, dependent on a combination of its previous experience, hormone levels and genetic makeup (Rubenstein & Hauber, 2008). On the other hand, a more ornamented bird may be exposed to more aggressive behavior by conspecifics, and the costs it will pay may depend on its individual susceptibility to such aggressiveness, or its individual ability to cope with higher hormone levels (Kempnaers *et al.*, 2008; Safran *et al.*, 2008; Rubenstein & Hauber, 2008). This may provide us with a possible explanation for the costs of being colorful in godwits, but also makes it clear that genotype and environment both affect godwit plumage ornamentation. In black-tailed godwits, there is covariation of microsatellites repetitions on the POMC gene with male plumage ornamentation (Figure 10.4, A. Roulin, pers. comm.), supporting that this system might be involved in plumage expression.

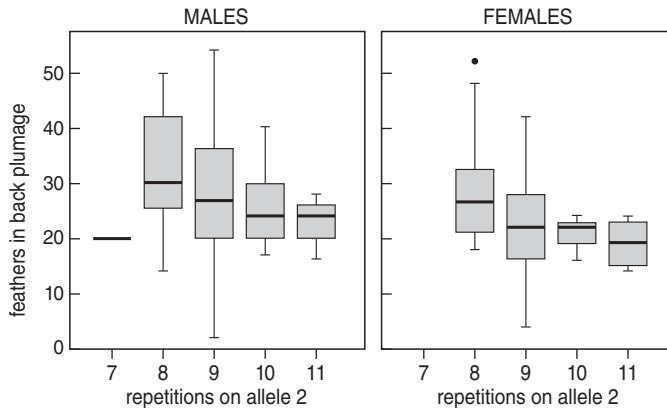


Figure 10.4: The number of feathers in the back plumage of black-tailed godwits breeding in The Netherlands in relation to microsatellite repetitions on the *pomc*-gene. Preliminary data provided by A. Roulin based on samples from our study population.

We have some, albeit anecdotal, support for a link between plumage coloration and aggressiveness: Godwits spend late winter and early spring on the Iberian Peninsula, where they moult into breeding plumage. We carried out an experiment during spring staging on captive black-tailed godwits (*Box C*). Captive godwits were fed rice or fly larvae *ad libitum* for six weeks during spring staging. We found that although the birds feeding on fly larvae fattened up quickly, they did not moult into a colorful breeding plumage. Birds feeding on rice, at excess availability, did only gain little mass, but (mostly males) moulted in a more ornamented and colorful breeding plumage (*Box C*). The captive birds feeding on rice often displayed aggressive behavior towards each other, while this did not occur in the birds feeding on fly larvae. It is likely that such behavior is indicative of, or induces, stress and therewith may be related with steroid levels (Soma, 2006) – traits that due to the pleiotropic effects are likely related to the expression of melanin-based plumage traits (Ducrest *et al.*, 2008).

Thus in our experiments, it is not possible to distinguish whether birds were aggressive towards each other in the first place, and lost weight because of the frequent aggressive and presumably energy consuming interactions, or whether they lost weight because they were eating rice, leading to a worse nutritional condition which in turn caused stress that could have induced aggressiveness. However, since such aggressive interactions are likely to incur severe physiological costs, this may have led to the lower body mass, which represent some of the costs for being colorful (Ketterson & Nolan, 1999; Wikelski *et al.*, 1999; Owen-Ashley, Hasselquist & Wingfield, 2004; Safran *et al.*, 2008).

Further, although we could show that melanin ornamentation in godwits has a genetic basis (Chapter 8 and Figure 10.4), we also showed that there is some phenotypic plasticity (*Box C*). We do not know how exactly the interplay between genotype and environment affects godwit plumage ornamentation. Nevertheless, the experi-

ment does provide anecdotal support for a link between aggressiveness, plumage ornamentation and body mass change in male godwits.

The benefits of being pale

The assumption of a link between intraspecific aggression and plumage coloration in male godwits allowed us to formulate further expectations on why it might be beneficial for a male godwit to be pale. Given that more colorful male godwits are more aggressive towards conspecifics, we expect them to be more successful when interacting with conspecifics and drive them away from their preferred nesting site (Andersson, 1994; McGraw *et al.*, 2003). Therefore, we expect paler males to breed in higher densities than more ornamented males. Since we know that on agricultural land paler godwit males are more successful than more ornamented males (Chapter 5), we expect pale male godwits of nests in higher densities to have an advantage (their nests containing larger eggs or being more likely to hatch) over the ones breeding more solitary and being more colorful. One mechanism for this advantage could be that high nesting densities could be linked with a more effective anti-predation behavior (Elliot, 1985).

Black-tailed godwits employ a group defense against aerial and ground predators, where a number of godwits flocks together and harasses the intruder (Beintema *et al.*, 1995). When engaging in anti-predation behavior parents increase the chances of survival of their young, but this comes at costs for the parents. Although the chance of the adult being killed or injured by the predator seems to be low, it exists (Sordahl, 1990). This chance is likely lower when a large number of birds engage in the defense than when it is done by a single bird only, because the risk of being killed is spread among a larger number of birds and, more importantly, because a nest defense with a large number of participants can reduce the overall rate of predator visits (Elliot, 1985).

In areas with a high density of nests, such flocks of defending godwits may consist of a large number of godwits, simply because many birds are available. It may therefore be beneficial to breed in higher densities because the number of birds that participate in such a defense is higher, which should ultimately lead to a higher breeding success (for example Becker, 1995; Larsen *et al.*, 1996; Harris 2008). We also expect that nests that are defended by a larger number of birds to contain larger eggs, because females may be more prone to invest into eggs if they are likely to be more secure. Another expectation is that these eggs are more likely to hatch, because predation defense is more effective.

If it is true that in godwits plumage coloration and aggressiveness are linked, we speculate that male godwits with a more colorful plumage may be more successful in competitive interactions with conspecifics. In non-agricultural settings, a trade-off between a large territory around the nest site and breeding in high densities may have existed. In a natural habitat, more ornamented male godwits may have been able to secure a larger, and presumably better, territory whose resources need not to be shared with others, benefiting the territory owner and his mate (McGraw *et al.*, 2003). The

costs of having a large territory would have been a less effective predator defense. In areas where food availability is not constrained and predation pressure increased, like the current breeding habitat of godwits (see above), it may be that this trade-off has changed, with food availability and the size of the territory being less important and predator defense being more important.

In Chapter seven we show that paler males breed in higher densities, while more ornamented males bred more solitarily, indicating that they were either able to evict other godwits from their territory, or that paler males preferred nesting sites in higher densities. However, our data suggests that the nesting sites of males with more colorful breeding plumage are of lower quality. Breeding success with respect to egg volume and hatching success was lower (Chapter seven). The explanation for this may be that the quality of a nesting site is mostly determined by nest density and predator defense and only to a lesser extent by other local characteristics of that site, like food availability. One complication of this hypothesis is that not much is known about nesting densities of *limosa* godwits in non-agricultural areas. Haverschmidt (1963) notes about godwits breeding in a moor, that "Godwits like to nest in each others company, and sometimes we can even speak of colonies." And: "[...] this is not the general rule and many isolated pairs occur". If we infer that nesting densities in bog and fen areas resembled nesting densities as they occur in the *islandica* subspecies on Iceland, godwit in the past in natural areas likely bred in lower densities than they do nowadays in grassland.

However, our interpretation hinges on the assumption that habitat quality in terms of food availability for adults is relatively high, and that adult food-related differences between territories have little biological meaning. One could also think of the possibility that areas where godwits breed in higher densities are of considerably high quality and can harbor a much larger number of godwits than areas with solitary nests. If this would be true, we cannot explain why more ornamented godwits would not breed in these high quality areas, too. The assumption that agricultural areas provide ample food supply for adults as opposed to the natural breeding habitat of godwits, marshes and bogs was made before (Bijlsma *et al.*, 2001; Zwarts *et al.*, 2009). After moving the breeding habitat from natural areas into the agricultural grassland, godwits and other meadowbirds that relied on similar food experienced a considerable population increase, which is usually explained by the better food availability (Beintema *et al.*, 1995; Bijlsma *et al.*, 2001; Zwarts *et al.*, 2009). Second, experimental studies have shown that an increase in food availability can enable birds to lay their eggs earlier in the season (see review by Meijer & Drent, 1999). When the usage of soil fertilizer increased during the last half of the last century, many meadowbirds including black-tailed godwits, shifted their laying date forward (Beintema *et al.*, 1985). It was suggested that a nitrogen-related increase in food availability enabled black-tailed godwits to lay their clutches two weeks earlier (Beintema *et al.*, 1985; Zwarts *et al.*, 2009). This leads us to believe that it is valid to assume that food availability for adult godwits in agricultural grasslands is not a constraining factor for the choice of a nesting site.

We conclude that two factors, higher food availability and predation pressures,

could have decreased the benefits of obtaining a larger, solitary nesting site for black-tailed godwits breeding in agricultural grasslands. Further, higher food availability may have enabled godwits to breed at higher densities where they could meet the dangers of increased predation pressures more efficiently, benefiting less aggressive and paler male black-tailed godwits.

This does not contradict the results of Chapter 3, where we conclude that food availability for adults affects female reproductive investment. In Chapter 3 we found that godwits may allocate resources to eggs depending on how many resources they have at their disposal. We also found that nowadays, godwits invest more into reproduction early in the season than thirty years ago. This might imply that there are more resources to be allocated to reproduction than there may have been thirty years ago; suggesting that food availability early in the season may now be higher than it used to be. Since circumstances early in the season reflect circumstances during settlement, this supports our idea that a change in habitat quality may have been responsible for the decrease in sexual plumage dimorphism in black-tailed godwits.

We have collected circumstantial support for the notion that the change from natural breeding grounds towards agricultural grasslands, and the ongoing intensification of those grasslands, may have led to a decrease in black-tailed godwit sexual plumage dimorphism. However, all our results are correlational, and we do not have proof of causality. Further, we cannot distinguish whether pale males are pale and more ornamented males are more ornamented because of their genetic make-up, or whether these differences are due to phenotypic plasticity. In this thesis, we found some evidence for a genetic basis of male plumage coloration (Chapter 8, Figure 9.4). On the other hand, we also showed that plumage expression might be a phenotypical effect (*Box C*). For a better understanding of the change in plumage traits in godwits, we need to calculate heritability estimates, thus we need to undertake cross-foster and diet experiments to find out how much of this variation is determined by genotype and how much by environmental variation. All this is currently not an option in Black-tailed godwits, since it requires a much larger dataset, and more importantly, more data on recruits which is not available due to the low productivity.

Predicting the fall of the black-tailed godwit in The Netherlands

Accepted for publication in Dutch in *De Levende Natuur*

MY COAUTHORS and I investigated individual fitness correlates of black-tailed godwits. I tried to take a historical perspective to find out about current and past selection pressures acting on godwits that may affect population growth rates. In the light of the demands of agricultural politics and economics, it is questionable if any of this can help stop or slow down the decline of the black-tailed godwit in The Netherlands. In this last part, I want to look at population dynamics. By now there is ample empirical data on basic demographic variables available. I will use these and set up simple population dynamics models to predict how long it will take long until the godwit,

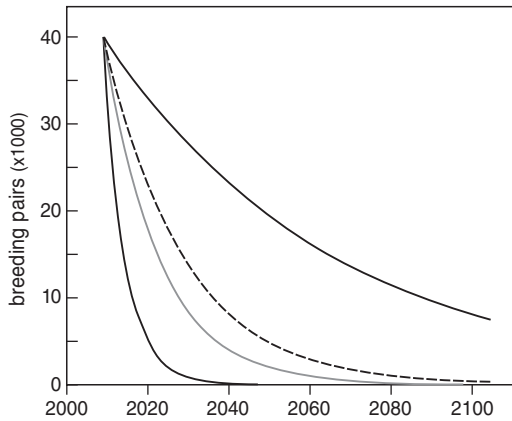


Figure 10.5: Predicted development of the number of breeding pairs of black-tailed godwits in The Netherlands. Left line assumes an adult survival of 0.77 (van Noordwijk & Thomson, 2008) and an annual reproductive success of 0.3 fledged young per pair; right line an adult survival of 0.85 (Roodbergen *et al.*, 2008) and an annual reproductive success of 0.7 fledged young per pair (Schekkerman *et al.*, 2008). Population dynamics were calculated following equation (1), assuming that 100% of all present birds proceed to breed. The grey line depicts a scenario with high reproduction (0.7) and high adult survival (0.85), but where only 60% of the population actually proceeds to breed. Dashed line depicts a decline of 5% annually.

formerly the “King of the meadow birds” (Thijssse, 1904), will be a rare –or even extinct– breeding bird in The Netherlands.

Currently, the Dutch godwit population is declining at a rate of 5% per annum (Gill *et al.*, 2008; Zwarts *et al.*, 2009). This decline started in the 1970s, from a maximum population size of 135 000 breeding pairs (Bijsma *et al.*, 2001; Gill *et al.*, 2008). Currently, there are an estimated 40 000 godwit pairs breeding in The Netherlands (Zwarts *et al.*, 2009). If we simply assume the population will continue to decrease by 5% annually, there will be less than 15000 pairs by 2030 (Fig. 10.5, dotted line). Similar to what I did in Schroeder *et al.* (2008b), I calculated the population development using a more accurate mathematical model. This model is not intended to replace an analysis of population viability (PVA), however, it still can serve as an eye-opener for how the future will look like, and the model can highlight actions that are long overdue to be taken.

$$\text{Population size} = \text{surviving adults} + \text{surviving fledglings last year}$$

I assume survival in the first year after fledging to be lower than in consecutive years ($s_{\text{juv}} < s_{\text{ad}}$). Also, I assume that only 20% (p) of all birds start breeding in their second calendar year, and the remaining 80% only start breeding in the third calendar year. The population at a given time (t) consists of the following:

$$(1) \quad N(t) = N_{CY2}(t) + N_{ad}(t)$$

One year later, there will be new offspring. A constant reproductive success ($r = 0.5$ * fledglings produced per pair) gives:

$$N_{CY2}(t+1) = s_{juv}(N_{CY2}(t) \cdot r \cdot p + N_{ad}(t) \cdot r)$$

Plus the remaining, surviving birds:

$$s_{ad} (N_{CY2}(t) + N_{ad}(t))$$

Thus the total population one year later will be the sum of the latter two:

$$N(t+1) = s_{juv}(N_{CY2}(t) \cdot r \cdot p + N_{ad}(t) \cdot r) + s_{ad} (N_{CY2}(t) + N_{ad}(t))$$

$$N(t+1) = s_{juv} \cdot r \cdot p \cdot N_{CY2}(t) + s_{juv} \cdot r \cdot N_{ad}(t) + s_{ad} (N_{CY2}(t) + N_{ad}(t))$$

Plugging (1) in the latter equation gives me:

$$(2) \quad N(t+1) = s_{juv} \cdot r \cdot p \cdot N_{CY2}(t) + s_{juv} \cdot r \cdot N_{ad}(t) + s_{ad} \cdot N(t)$$

For the sake of simplicity I assume no density dependence. I use this model (2) to predict the population development from 2009 onwards, starting with a population size of 40 000 breeding pairs, and a starting population of second year birds ($N_{CY2}(1) = N(1) \cdot r \cdot s_{juv}$). I set the fledgling survival rate of the first year to be 0.4, and all consecutive survival to 0.77 (van Noordwijk & Thompson, 2008) or 0.85 (Roodbergen *et al.*, 2008). Observed estimates of reproductive productivity have been shown to range from averages of 0.7 to 0.3 fledged young per pair and year (Schekkerman *et al.*, 2008). Figure 10.5 shows the most conservative (adult survival = 0.77; reproductive success = 0.3) and the most optimistic prediction (adult survival = 0.85; reproductive success = 0.7). Under the best circumstances, the Dutch godwit population will continue to decline, reaching less than 15 000 breeding pairs by the year 2060, while a more conservative estimate predicts this to happen within the next 5 years.

This rather dark outlook is actually an optimistic version of reality. First of all, average reproductive success of 0.7 has only been observed in the eighties, while more current estimates barely reach averages of 0.3 fledged young per pair and year, more often than not productivity is close to zero (Schekkerman *et al.*, 2008). Therefore, the more conservative, “bad” outcome is expected to be closer to reality. Furthermore, a large proportion of adult birds do return to the breeding grounds, but do not proceed to breed. In the Workumerwaard – an area managed explicitly for meadowbirds – the percentage of birds that return to the breeding site but do not proceed to breed was between 11-50% in the years 2006-2008 (P.M. Lourenço, pers. comm.; own observation). I incorporate this in the equation by adding a parameter b (proportion of

breeding birds), and set it to 60%:

$$(3) \quad N(t+1) = b (s_{\text{juv}} r p N_{\text{CY2}}(t) + s_{\text{juv}} r N_{\text{ad}}(t)) + s_{\text{ad}} N(t)$$

This darkens the picture considerably. Under the best conditions (high adult survival and high reproductive output), the Dutch breeding population of black-tailed godwits drops under 10 000 breeding pairs as early as 2030 (Fig. 10.5, grey line).

To improve the situation of the black-tailed godwit in The Netherlands, we can either aim to improve survival or productivity. Survival is hard to modify, given the already very high annual survival of godwits and the relatively efficient hunting ban. Therefore I aim at predicting how much productivity has to improve to *stabilize* the population decline. Productivity is determined by two components: the proportion of birds actually proceeding to breed (b), and the actual number of fledged young per pair and year (r ; reproductive output). In addition to aiming to increase productivity, we can also try and improve habitat quality, to increase the proportion of birds proceeding to breed each year. I use equation (3) to estimate the necessary reproduction rate (r , in fledged young per breeding pair) of godwits in The Netherlands in order to achieve a *stable, self-sustaining population* with respect to values for the proportion of the population breeding (b) ranging from 40% to 100% (all birds breed that year), incrementing at 10% each step. I did this for both, high and low adult annual survival estimates ($s_{\text{ad}} = 0.77$ and $s_{\text{ad}} = 0.85$). Fig. 10.6 shows the results of this model. It becomes very clear that in order to halt the population decline, we have to improve

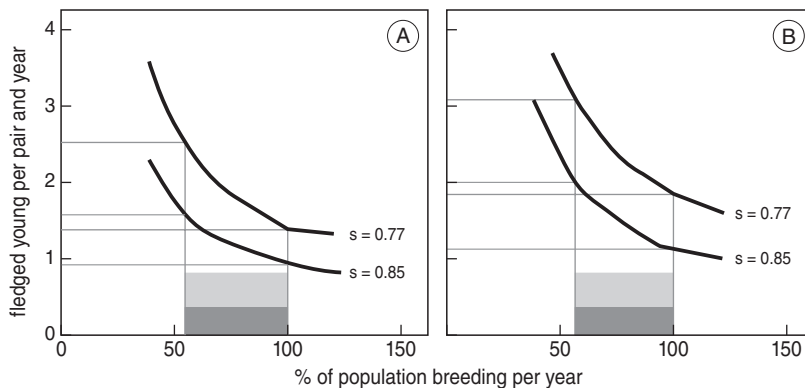


Figure 10.6: Model results depicting the necessary reproductive success for (A) a stable godwit population and (B) one that triples until 2025 in The Netherlands, in relation to the proportion of present birds proceeding to breed and adult survival rate. The dark grey shaded area depicts the realistic range of empirically observed values for both, the annual reproductive output and the proportion of returned birds that breed (Values >than 100% indicate that a given proportion of pairs need to breed twice in one season). The lighter shaded grey area depicts the range of annual reproductive success measured twenty years ago (Schekkerman *et al.*, 2008). Dashed lines give the annual reproductive success necessary for a stable population in a scenario with realistic parameter values.

reproductive success considerably, and simultaneously aim at high proportions of breeding birds. Under the very best scenario, with an adult survival of 0.85 and all birds breeding every year from now on (which is likely to be optimistic in most cases), we have to amplify reproductive success to values of at the very least 0.85 fledged young per pair and year.

In order to stabilize the population, we have to improve reproductive success to even higher levels than those measured thirty years ago; while in the meantime, reproductive success has been declining over the years (see above; Schekkerman *et al.*, 2008; H. Hötter, pers. comm.). Further, our lowest assumed value of reproductive output, 0.3 fledged young per pair and year, is a rather optimistic estimate as an average for the whole of The Netherlands. As the studies measuring this value did not take a proportion of non-breeders into account the actual value of fledged young *per pair, including non-breeders*, per year is likely lower than the assumed one. Breeding success in the Workumerwaard has been considerably below 0.3 during the five years while I did field work there (own observation). If this is representative for other meadowbird reserves, and if these values are generally higher than those of non-reserve areas, the increase in reproductive success of all godwit pairs breeding in The Netherlands will have to be considerably larger in order to only keep the population stable. Note that the Friesian provincial government has announced the ambitious plan to increase the godwit population threefold by 2025. To achieve this, every godwit pair breeding in Friesland has to successfully fledge at the very least 1.2 chicks every year from now onwards until 2025, assuming that every bird that returns to Friesland breeds and has a high adult survival. For other scenarios, the value rises to 3 and more fledged young per pair and year. It is obvious that it is close to impossible to take such measures. However, although our goal to stop the decline is very ambitious and heartening, it is not a realistic one if no severe measures are taken.

Measures taken by the Dutch government in the past have had little effect, and were and still are expensive (although not so in comparison with the enormous sums of financial support expanded on our agro-industry through EC's agricultural policies!). Warnings that meadowbirds are in decline have been issued since the early 1980ies. Furthermore, in 2001, David Kleijn and colleagues showed that "Agri-environment schemes do not effectively protect biodiversity in Dutch agricultural landscapes"; godwits and other meadowbirds did not breed more frequently in areas that received special treatment (Kleijn *et al.*, 2001, Kleijn & van Zuijlen, 2004). The Dutch government spends on average five times more subsidies per hectare for intensively managed areas where breeding densities and success are low, than on areas managed specifically for meadowbirds, where the actual measures taken are much better although still not enough to halt the decline (T. Piersma, pers. comm.). It is clear that taxpayer's money needs to be spent in ways that actually improve the situation for meadowbirds.

Consequently, if we are honest about stopping the decline, we have to take severe measures to ensure high productivity. Such measures need to encompass the creation of large nature reserves managed explicitly for meadowbirds, regardless of economic benefits by agriculturally exploiting the grassland. It has become crystal clear during

the last five years that we need to maximize chick survival in order to increase breeding success (Schekkerman & Beintema, 2007, Schekkerman *et al.*, 2008; Schekkerman 2008). Water management has to be adjusted to increase water levels in order to improve the food situation for adults and chicks (Verhulst *et al.*, 2007). But most importantly, mowing needs to be postponed until after all chicks fledged (Schekkerman & Beintema, 2007; Schekkerman *et al.*, 2008). This would improve chick survival considerably and in addition extend the breeding season. Currently, godwits leave very soon after mowing starts, which leads to a shortened breeding season and a low breeding success of the population (Zwarts *et al.*, 2009). More than half a decade ago, “the ordinary hay harvest [i.e. non-mechanical mowing, which was predominant at that time, annotation by JS] is done for 78% after June, 1st” (Klomp, 1951). In order to reestablish a longer breeding season in which godwits have a chance at laying a second, or replacement clutch mowing needs to be postponed until July. In short, the solution to halt the decline of the black-tailed godwit is straightforward in theory, but the realization of it will be difficult, as it has to be negotiated with respect to economical expectations of grassland owners. But if we want change (something e.g. the Netherlands Ministry of Agriculture has pledged more than once), we must act fast and strongly, or it will be too late.

References

A

- Akaike, H. (1973) Information theory and an extension of the maximum likelihood principle. Proceedings of the Second International Symposium on Information Theory. Akademiai Kiado, Budapest.
- Almasi, B., Roulin, A., Jenni-Eiermann, S. & Jenni, L. (2008) Parental investment and its sensitivity to corticosterone is linked to melanin-based coloration in barn owls. *Hormones and Behavior* 54, 217–223.
- Amat, J. A., Fraga, R. M. & Arroyo, G. M. (2001) Variations in body condition and egg characteristics of female Kentish plovers *Charadrius alexanderinus*. *Ardea* 89, 293–299.
- Andersson, M. (1994) Sexual Selection. Princeton University Press, Princeton, N.J.

B

- Baker, A. J., González, P. M., Piersma, T., Niles, L. J., de Serrano do Nascimento, I., Atkinson, P. W., Clark, N. A., Minton, C. D. T., Peck, M. K. & Aarts, G. (2004) Rapid population decline in red knots: fitness consequences of decreased refueling rates and late arrival in Delaware Bay. *Proceedings of the Royal Society London Series B* 271, 875–882.
- Baker, A. J., Huynen, L. J., Haddrath, O., Millar, C. D. & Lambert, D. M. (2005) Reconstructing the tempo and mode of evolution in an extinct clade of birds with ancient DNA: The giant moas of New Zealand. *Proceedings of the National Academy of Sciences USA* 102, 8257–8262.
- Bantock, T. M., Prys-Jones, R. P. & Lee, P. L. M. (2008) New and improved molecular sexing methods for museum bird specimens. *Molecular Ecology Resources* 8, 519–528.
- Bateman, A. J. (1948) Intra-sexual selection in *Drosophila*. *Heredity* 2, 349–368.
- Battley P., Dietz M. W., Piersma T., Dekinga A., Tang S. & Hulsman K. 2001. Is long-distance bird flight equivalent to a high-energy fast? Body composition changes in freely migrating and captive fasting great knots. *Physiological and Biochemical Zoology* 74, 435–449.
- Battley, P. F. (2007) Plumage and timing of migration in bar-tailed godwits: a comment on Drent et al., (2003). *Oikos* 116, 349–350.
- Battley, P. F., Rogers, D. I. & Hassel, C. J. (2006) Prebreeding moult, plumage and evidence for a presupplemental moult in the great knot *Calidris tenuirostris*. *Ibis* 148, 27–38.
- Battley, P. F., Piersma T., Rogers D. I., Dekinga A., Spaans B. & van Gils J.A. (2004) Do body condition and plumage during fuelling predict northwards departure dates of great knots *Calidris tenuirostris* from North-west Australia? *Ibis* 146, 46–60.
- Becker, P. H. (1995) Effects of coloniality on gull predation on common tern (*Sterna hirundo*) chicks. *Colonial Waterbirds* 18, 11–22.
- Becker, W. A. (1984) A Manual of Quantitative Genetics. Academic Enterprises, Pullman.
- Beintema, A. J. & Müskens, G.J.D.M. (1987): Nesting success of birds breeding in Dutch agricultural grasslands. *Journal of Applied Ecology* 24, 743–758.
- Beintema, A. J. & Visser, G. H. (1988a) Growth parameters in chicks of charadriiform birds. *Ardea* 77, 169–180.
- Beintema, A. J. & Visser, G. H. (1988b) The effect of weather on time budgets and development of chicks of meadow birds. *Ardea* 77, 181–191.
- Beintema, A. J., Beintema-Hietbrink, R. J. & Müskens, G. J. D. M. (1985) A shift in the timing of breeding in meadow birds. *Ardea* 73, 83–89.
- Beintema, A. J., Thissen, J. B., Tensen, D. & Visser, G. H. (1991) Feeding ecology of charadriiform chicks in agricultural grassland. *Ardea* 79, 31–44.
- Beintema, A. J. (1995) Fledging success of meadowbirds, estimated from ringing data. *Ringling & Migration* 16, 129–139.
- Beintema, A., Moedt, O. & Ellinger, D. (1995) Ecologische Atlas van de Nederlandse Weidevogels. Schuyt & Co, Haarlem
- Bennett, P. M. & Owens, I. P. F. (2002) Evolutionary Ecology of Birds – Life Histories, Mating Systems and Extinction. Oxford Series in Ecology and Evolution, Oxford University Press, Oxford.
- Bêty, J., Gauthier, G., & Giroux, J.F. (2003) Body condition, migration and timing of reproduction in snow geese. A test of the condition-dependent model of optimal clutch size. *American Naturalist* 162, 110–121.

- Bêty, J., Giroux, J.F., & Gauthier, G. (2004) Individual variation in timing of migration: causes and reproductive consequences in greater snow geese (*Anser caerulescens atlanticus*). *Behavioral Ecology and Sociobiology* 57, 1–8.
- Bijlsma, R. G., Hustings, F. & Camphuysen, C. J. (2001) *Algemene en Schaarse Vogels van Nederland (Avifauna van Nederland 2)*. GMB Uitgeverij/KNNV Uitgeverij, Haarlem/Utrecht.
- BirdLife International (2006). *Limosa limosa*. In: 2006 IUCN Red List of Threatened Species. URL: www.iucnredlist.org. Downloaded on 28 September 2008.
- Black, D. L. (2003) Mechanisms of alternative pre-messenger RNA splicing. *Annual Review of Biochemistry* 72, 291–336.
- Black, J. M. & Owen, M. (1995) Reproductive performance and assortative pairing in relation to age in barnacle geese. *Journal of Animal Ecology* 64, 234–244.
- Black, J. M. (ed.) (1996) *Partnerships in Birds. The Study of Monogamy*. Oxford University Press, Oxford.
- Blomqvist, D., Johansson, O. C. & Götmark, F. (1997) Parental quality and egg size affect chick survival in a precocial bird, the lapwing *Vanellus vanellus*. *Oecologia* 110, 18–24.
- Blomqvist, D., Kempenaers, B., Lanctot, R. B. & Sandercock, B. K. (2002) Genetic parentage and mate guarding in the arctic-breeding western sandpiper. *Auk* 119, 228–234.
- Bókonyi, V., Garamszegi, L., Hirschenhauser, K. & Liker, A. (2008) Testosterone and melanin-based black plumage coloration: a comparative study. *Behavioral Ecology and Sociobiology* 62, 1229–1238.
- Bolton M (1991) Determinants of chick survival in the Lesser black-backed gull: relative contributions of egg size and parental quality. *Journal of Animal Ecology* 60, 949–960.
- Bolton, M., Houston, D. & Monaghan, P. (1992) Nutritional constraints on egg formation in the lesser black-backed gull: An experimental study. *Journal of Animal Ecology* 61, 521–532.
- Bonato, M., Evans, M. & Cherry, M. (2009) Investment in eggs is influenced by male coloration in the ostrich, *Struthio camelus*. *Animal Behavior*, 77, 1027–1032.
- Both, C. & Visser, M. E. (2001) Adjustment to climate change is constrained by arrival date in a long-distance migrant bird. *Nature* 411, 296–298.
- Both, C. & Visser, M. E. (2005) The effect of climate change on the correlation between avian life history traits. *Global Change Biology* 11, 1606–1613.
- Both, C., Bouwhuis, S., Lessells, C. M., & Visser, M. E. (2006) Climate change and population declines in a long distance migratory bird. *Nature* 441, 81–83.
- Both, C., Piersma, T. & Roodbergen, S. P. (2005) Climate change explains much of the 20th century advance in laying date of northern lapwing *Vanellus vanellus* in The Netherlands. *Ardea*, 93, 79–88.
- Both, C., van Asch, M., Bijlsma, R. G., van den Burg, A. B., & Visser, M. E. (2009) Climate change and unequal phenological changes across four trophic levels: constraints or adaptations. *Journal of Animal Ecology* 78, 73–83.
- Boulinier, T., Danchin, E., Monnat, J.Y., Doutrelant, C. & Cadiou, B. (1996) Timing of prospecting and the value of information in a colonial breeding bird. *Journal of Avian Biology* 27, 252–256.
- Brinkhof, M. W. G., Cavé, A. J., Daan, S. & Perdeck, A. C. (2002) Timing of current reproduction directly affects future reproductive output in European coots. *Evolution* 56, 400–411.
- Brunton, D. H. 1990. The effects of nesting stage, sex, and type of predator on parental defense by killdeer (*Charadrius vociferous*): testing models of avian parental defense. *Behavioural Ecology and Sociobiology*, 26, 181–190.
- Bryk, A. S. & Raudenbush, S. W. (1992) *Hierarchical Linear Models*. Sage, Newbury Park, CA.
- Buratti, E. & Baralle, F. (2004) Influence of RNA secondary structure on the pre-mRNA splicing process. *Molecular and Cellular Biology* 24, 10505–10514.
- Burnham, K. P. & Anderson, D. R. (2002) *Model selection and multimodel inference*. Springer Verlag, New York.
- Burns, K.J. (1998) Aphylogenetic perspective on the evolution of sexual dichromatism in tanagers (Thraupidae): the role of female versus male plumage. *Evolution* 52, 1219–1224

C

- Cam, E., Link, W. A., Cooch, E. G., Monnat, J. Y. & Danchin, E. (2002) Individual covariation in life-history traits: Seeing the trees despite the forest. *American Naturalist* 159, 97–105.
- Carey, J. R. & Judge, D. S. (2001) Longevity records: Lifespans of mammals, birds, amphibians, reptiles and fish. *Monographs on Population Aging*. University Press of Southern Denmark, Odense.
- Carroll, S. P., Hendry, A. P., Reznick, D. N. & Fox, C. W. (2007) Evolution on ecological time-scales. *Functional Ecology* 21, 387–393.
- Casey, E. C., Jones, K. L., Sandercock, B. K. & Wisley, S. M. (2009) Heteroduplex molecules cause sexing errors in a standard molecular protocol for avian sexing. *Molecular Ecology Resources* 9, 61–65.
- Charmantier, A., McCleery, R. H., Cole, L. R., Perrins, C., Kruuk, L. E. B., & Sheldon, B. C. (2008) Adaptive phenotypic plasticity in response to climate change in a wild bird population. *Science* 320, 800–803.
- Choudhury, S. (1995) Divorce in birds: a review of the hypotheses. *Animal Behavior*. 50: 413–429.
- Cole, L. C. (1954) The population consequences of life history phenomena. *Quarterly Review of Biology* 29, 103–137.
- Cramp, S. & Simmons, K.E.L. (Eds) (1983) *Handbook of the Birds of Europe, the Middle East and North Africa. Volume III Waders to Gulls*. Oxford University Press, Oxford.
- Crawley, M. J. (2007) *The R Book*. Wiley, West Sussex.
- Cresswell, W. & McCleery, R. (2003) How great tits maintain synchronization of their hatch date with food supply in response to long-term variability in temperature. *Journal of Animal Ecology* 72, 356–366.
- Crick, H. Q. P. & Sparks, T. H. (1999) Climate change related to egg-laying trends. *Nature*, 399, 423–424.
- Crick, H. Q. P., Dudley, C., Glue, D. E., & Thomson, D. L. (1997) UK birds are laying eggs earlier. *Nature*, 388, 526–527.
- Cristol, D. A. (1995) Early arrival, initiation of nesting, and social status: an experimental study of breeding female red-winged blackbirds. *Behavioral Ecology*, 6, 87–93.
- Cunningham, E. J. A. & Russell, A. F. (2000) Egg investment is influenced by male attractiveness in the mallard. *Nature* 404, 74–77.

D

- Daan, S. & Tinbergen, J. M. (1997) *Adaptation of Life Histories*. In: *Behavioral Ecology* (eds. Krebs, J. R. & Davies, N. B.). Blackwell Publishing, Oxford.
- Daan, S., Dijkstra, C., & Tinbergen, J. M. (1990) Family planning in the kestrel (*Falco tinnunculus*): the ultimate control of covariation of laying date and clutch size. *Behavior* 114, 83–116.
- Dale, S., Steifetten, Ø., Osiejuk, T.S., Losak, K. & Cygan, J.P. (2006) How do birds search for breeding areas at the landscape level? Interpatch movements of male ortolan buntings. *Ecography* 29, 886–898.
- Danchin, E., Boulinier, T. & Massot, M. (1998) Conspecific reproductive success and breeding habitat selection: implications for the study of coloniality. *Ecology* 79, 2415–2428.
- Danchin, E., Giraldeau, L.A., Valone, T.J. & Wagner, R.H. (2004) Public information: From nosy neighbors to cultural evolution. *Science* 305, 487–491.
- Darwin, C. (1871) *The descent of man and selection in relation to sex*. Princeton University Press, Princeton, N.J.
- Dawkins, R. (1996) *The Blind Watchmaker*. Norton, New York.
- Dawson, D. A., Darby, S., Hunter, F.M., Krupa, A. P., Jones, I. L. & Burke, T. (2001) A critique of avian CHD-based molecular sexing protocols illustrated by a Z-chromosome polymorphism detected in auklets. *Molecular Ecology Notes* 1, 201–204.
- Delhey, K., Peters, A., Johnsen, A. & Kempenaers, B. (2007) Fertilization success and UV ornamentation in blue tits *Cyanistes caeruleus*: correlational and experimental evidence. *Behavioral Ecology* 18, 399–409.
- Dennett, C. D. (1995) *Darwin's dangerous idea: evolution and the meanings of life*. Simon & Schuster, New York.

- Denton, M.T., Schmidt, S., Critchley, C., & Stewart, G. R. (2001) Natural abundance of stable carbon and nitrogen isotopes in *Cannabis sativa* reflects growth conditions. *Australian Journal of Plant Physiology* 28, 1005–1012.
- Dobzhansky, T. G. (1973) Nothing in Biology makes sense except in the Light of Evolution. *American Biology Teacher* 35, 125–129.
- Doucet, S. M. & Hill, G. E. (2009) Do museum specimens accurately represent wild birds? A case study of carotenoid, melanin, and structural colours in long-tailed manakins *Chiroxiphia linearis*. *Journal of Avian Biology* 40, 146–156.
- Doucet, S. M. & Montgomerie, R. (2003) Multiple sexual ornaments in satin bowerbirds: ultraviolet plumage and bowers signal different aspects of male quality. *Behavioral Ecology* 14, 503–509.
- Drent, R. H. & Daan, S. (1980) The prudent parent: energetic adjustments in avian breeding. *Ardea* 68, 225–252.
- Drent, R. H. (2006) The timing of birds' breeding seasons: the Perrins hypothesis revised especially for migrants. *Ardea* 94, 305–322.
- Drent, R., Both, C., Green, M., Madsen, J. & Piersma, T. (2003) Pay-offs and penalties of competing migratory schedules. *Oikos* 103, 274–292.
- Drent, R.H., Fox, A.D. & Stahl, J. (2006) Travelling to breed. *Journal of Ornithology* 147, 122–134.
- Ducrest, A.-L., Keller, L. & Roulin, A. (2008) Pleiotropy in the melanocortin system, coloration and behavioral syndromes. *Trends in Ecology and Evolution* 23, 502–510.
- Dunn, P. O. (2004) Breeding dates and reproductive performance. *Advances in Ecological Research*, 35, 69–87.
- E**
- Ebbinge, B. S. & Spaans, B. (1995) The importance of body reserves in spring staging areas in the temperate zone for breeding in dark-bellied brent geese *Branta b. bernicla* in the high arctic. *Journal of Avian Biology* 26, 105–113.
- Edwards, C. A. & Lofty, J. R. (1982) Nitrogenous fertilizers and earthworm populations in agricultural soils. *Soil Biology & Biochemistry* 14, 515–521.
- Eldredge, N. & Gould, S. J. (1972) Punctuated equilibria: an alternative to phyletic gradualism. In Schopf, T.J.M. (Ed.) *Models in Paleobiology*. 82–115. San Freeman Cooper, San Fransisco.
- Ellegren, H. & Sheldon, B. C. (1997) New tools for sex identification and the study of sex allocation in birds. *Trends in Ecology & Evolution* 12, 255–259.
- Ellegren, H. (1996) First gene on the avian W chromosome (CHD) provides a tag for universal sexing of non-ratite birds. *Proceedings of the Royal Society London Series B*, 263, 1635–1641.
- Elliot R. D. (1985) The exclusion of avian predators from aggregations of nesting lapwings (*Vanellus vanellus*). *Animal Behavior* 33, 308–314.
- Endler, J. A. (1983) Natural selection and sexual selection on color patterns in poeciliid fishes. *Environmental Biology of Fishes* 9, 173–190.
- Engelmoer, M., Roselaar, C. S., Boere, G. C. & Nieboer, E. (1983) Post- mortem changes in measurements of some waders. *Ring and Migration* 4, 245–249.
- Erikstad, K. E., Fauchald, R., Tveraa, T. & Steen, H. (1998) On the cost of reproduction in long-lived birds: the influence of environmental variability. *Ecology* 79, 1781–1788.
- F**
- Falconer, D. S. & Mackay, T. F. (1996) *Introduction to quantitative genetics*. Longman, Harlow.
- Farmer, A. H. & Wiens, J. A. (1999) Models and reality: Time-energy trade-offs in pectoral sandpiper (*Calidris melanotos*) migration. *Ecology* 80, 2566–2580.
- Fasola, M., Zhang, Y., Zhao, D., Dong, Y. & Wang, H. (2001) Age-assortative mating related to reproductive success in black-crowned night herons. *Waterbirds*. 24, 272–276.
- Figuerola, J. (1999) A comparative study on the evolution of reversed size dimorphism in monogamous waders. *Biological Journal of the Linnean Society* 67, 1–18.
- Fisher, R. A. (1958) The genetical theory of natural selection. In: Bennet, H. (Ed) *The Genetical theory of natural selection*. A complete variorum Edition (1999) Oxford University Press, Oxford.
- Flynn, L., Nol, E. & Zharikov, Y. (1999) Philopatry, nest-site tenacity, and mate fidelity of Semipalmated Plovers. *Journal of Avian Biology* 30, 47–55.

- Fretwell, S. D. & Lucas, H. L. (1970) On territorial behavior and other factors influencing habitat distribution in birds. I. Theoretical Development. *Acta Biotheoretica*, 19, 16–36.
- Fridolfsson, A. K., & Ellegren, H. (1999) A simple and universal method for molecular sexing of non-ratite birds *Journal of Avian Biology* 30, 116–121.
- G**
- Gabriel, K. R. (1971) The biplot graphic display of matrices with application to principal components analysis. *Biometrika* 58, 453–467.
- Gelman, A. & Hill, J. (2006) *Data analysis using regression and multilevel/hierarchical models*. Cambridge University Press, Cambridge.
- Gerritsen, G. J. & Groen, N. M. (1995) Icelandic black-tailed godwit project 1993. Foundation Working Group For International Waterbird and Wetland Research (WIWO), WIWO-report 51, Zeist, The Netherlands.
- Gienapp, P., Teplitsky, C., Alho, J. S., Mills, J. A. & Merilä, J. (2008). Climate change and evolution: disentangling environmental and genetic responses. *Molecular Ecology* 17, 167–178.
- Gill, J. A., Langston, R. H. W., Alves, J. A., Atkinson, P. W., Bocher, P., Cidraes Vieira, N., Chrockford, N. J., Gélinaud, G., Groen, N., Gunnarsson, T. G., Hayhow, B., Hooijmeijer, J., Kentie, R., Kleijn, D., Lourenço, P., Masero, J. A., Meunier, F., Potts, P., Roodbergen, M., Schekkerman, H., Schroeder, J., Wymenga, E. & Piersma, T. (2007) Contrasting trends in two black-tailed godwit populations: A review of causes and recommendations. *Wader Study Group Bulletin* 114, 43–50.
- Gill, J. A., Norris, K., Potts, P. M., Gunnarsson, T. G., Atkinson, P. W. & Sutherland, W.J. (2001): The buffer effect and large-scale population regulation in migratory birds. - *Nature* 412: 436–438.
- Glutz von Blotzheim, U.N., Bauer, K.M. & Bezzel, E. (1985) *Handbuch der Vögel Mitteleuropas Band 7: Charadriiformes (2. Teil) Schnepfen-, Möven- und Alkenvögel*. Aula Verlag, Wiesbaden.
- Grafen, A. (1990) Biological signals as handicaps. *Journal of Theoretical Biology*, 144, 517–546.
- Green, R. E., Hiron, G. J. M. & Kirby, J. S. (1990) The effectiveness of nest defense by black-tailed godwits *Limosa limosa*. *Ardea*, 78, 405–413.
- Greenberg, R. & Marra, P. P. (Eds.) (2004) *Birds of two worlds: the ecology and evolution of migration*. The John Hopkins University Press, Baltimore.
- Griffith, S. C., Parker, T. H. & Olson, V. A. (2006) Melanin- versus carotenoid-based sexual signals: Is the difference really so black and red? *Animal Behavior* 71, 749–763.
- Griffith, S., Owens, I. & Burke, T. (1999) Female choice and annual reproductive success favour less ornamented male house sparrows. *Proceedings of the Royal Society London Series B* 266, 765–770.
- Griffiths, R., Daan, S. & Dijkstra, C. (1996) Sex identification in birds using two CHD genes. *Proceedings of the Royal Society London Series B* 263, 1251–1256.
- Griffiths, R., Double, M. C., Orr, K., & Dawson, R. J. G. (1998) A DNA test to sex most birds. *Molecular Ecology* 7, 1071–1075.
- Groen, N. M. & Hemerik, L. (2002) Reproductive success and survival of black-tailed godwits *Limosa limosa* in a declining local population in The Netherlands. *Ardea* 90, 239–248.
- Groen, N. M. & Yurlov, A. K. (1999) Body dimensions and mass of breeding and hatched black-tailed godwits (*Limosa l. limosa*): a comparison between a West Siberian and a Dutch population. *Journal of Ornithology* 140, 73–79.
- Groen, N. M. (1993) Breeding site tenacity and natal philopatry in the black-tailed godwit *Limosa l. limosa*. *Ardea* 81, 107–113.
- Gunnarsson, T. G., Gill, J. A., Sigurbjörnsson, T. & Sutherland, W.J. (2004) Arrival synchrony in migratory birds. *Nature* 431, 646.
- Gunnarsson, T. G., Gill, J. A., Petersen, A., Appleton, G. F. & Sutherland, W. J. (2005a) A double buffer effect in a migratory shorebird population. *Journal of Animal Ecology* 74, 965–971.
- Gunnarsson, T. G., Gill, J. A., Newton, J., Potts, P. M. & Sutherland, W. J. (2005b) Seasonal matching of habitat quality and fitness in a migratory bird. *Proceedings of the Royal Society London Series B* 272, 2319–2323.
- Gunnarsson, T. G., Gill, J. A., Goodacre, S. L., Gélinaud, G., Atkinson, P. W., Hewitt, G. M.,

- Potts, P. M. & Sutherland, W. J. (2006a) Sexing of black-tailed godwits *Limosa limosa islandica*: a comparison of behavioral, molecular, biometric and field-based techniques. *Bird Study* 53, 193–198.
- Gunnarsson, T. G., Gill, J. A., Atkinson, P. W., Gelinaud, G., Potts, P., Croger, R. E., Gudmundsson, G. A., Appleton, G. F., & Sutherland, W. J. (2006b) Population-scale drivers of individual arrival times in migratory birds. *Journal of Animal Ecology*, 75, 1119–1127.
- Gunnarsson, U., Hellström, A. R., Tixier-Boichard, M., Minvielle, F., Bed'hom, B., Ito, S., Jensen, P., Rattink, A., Vereijken, A. & Andersson, L. (2007) Mutations in SLC45A2 cause plumage color variation in chicken and Japanese quail. *Genetics*, 175, 867–877.
- H**
- Hammock, E. & Young, L. (2005) Microsatellite instability generates diversity in brain and sociobehavioral traits. *Science* 308, 1630–1634.
- Harris, M. P. (2008) Breeding performance of puffins *Fratercula arctica* in relation to nest density, laying date and year. *Ibis* 122, 193–209.
- Harshman, L. G. & Zera, A. J. (2007) The costs of reproduction: the devil in the details. *Trends in Ecology and Evolution* 22, 80–86.
- Haverschmidt, F. (1963) The black-tailed godwit. E. J. Brill, Leiden.
- Hegener, R. E. & Wingfield, J. C. (1987) Effects of experimental manipulation of testosterone levels on parental investment and breeding success. *Auk* 104, 462–469.
- Hegyi, Z. & Sasvári, L. (1998) Components of fitness in lapwings *Vanellus vanellus* and black-tailed godwits *Limosa limosa* during the breeding season: do female body mass and egg size matter? *Ardea* 86, 43–50.
- Heinsohn R., Legge S. & Endler J. A. (2005) Extreme reversed sexual dichromatism in a bird without sex role reversal. *Science* 309, 617–619.
- Hill, G. E. & McGraw, K. J. (2006a) Bird Coloration, Volume 1, Mechanisms and Measurements. Harvard University Press, Cambridge (MA).
- Hill, G. E. & McGraw, K. J. (2006b) Bird Coloration, Volume 2, Function and Evolution. Harvard University Press, Cambridge (MA).
- Hill, G. E. (2002) Red bird in a brown bag: The function and evolution of colorful plumage in the house finch. Oxford University Press, New York.
- Hobson, K.A. & Clark, R.G. (1992) Assessing avian diets using stable isotopes. II: Factors influencing diet-tissue fractionation. *The Condor* 94, 189–197.
- Hobson, K.A. (1999) Tracing origins and migration of wildlife using stable isotopes: a review. *Oecologia* 120, 314–326.
- Hochachka, W.W. (1990) Seasonal decline in reproductive performance of song sparrows. *Ecology* 71, 1279–1288.
- Höglund, J., Johansson, T., Beintema, A. & Schekkerman, H. (2009) Phylogeography of the black-tailed godwit *Limosa limosa*: substructuring revealed by mtDNA control region sequences. *Journal of Ornithology* 150, 45–53.
- Hötter, H., Jeromin, H. & Melter, J. (2007) Brutbestände der Wiesen-Limikolen in Deutschland – Ergebnisse des Zählgebietsmonitorings. *Vogelwelt*, 2, 49–65.
- I**
- Inman, A. J. & Krebs, J. (1987) Predation and group living. *Trends in Ecology and Evolution*, 2, 31–32.
- J**
- Jackson, D.B. (1994) Breeding dispersal and site-fidelity in three monogamous wader species in the Western Isles, UK. *Ibis* 136, 463–473.
- Jawor, J. M. & Breitwisch, R. (2003) Melanin ornaments, honesty, and sexual selection. *Auk* 120, 249–265.
- Jawor, J. M. (2007) Testosterone in northern cardinals (*Cardinalis cardinalis*): possible influence of prolonged territorial behavior. *Auk* 124, 331–338.
- Johnson, D. H. (1979) Estimating Nest Success: The Mayfield method and an alternative. *The Auk* 96, 651–661.
- Johnson, P. J. & Lanyon, S. M. (2000) Evolutionary changes in color patches of blackbirds are associated with marsh nesting. *Behavioral Ecology*, 11, 515–519.

- Jönsson, P. E. & Alerstam, T. (1990) The adaptive significance of parental role division and sexual size dimorphism in breeding shorebirds. *Biological Journal of the Linnean Society* 41, 301–314.
- Jordan, D., Miles, R. J., Hubbard, V. C. & Lorenz, T. (2004) Effect of management practices and cropping systems on earthworm abundance and microbial activity in Sanborn field: a 115-year-old agricultural field. *Pedobiologia* 48, 99–110.
- Jukema, J. & Piersma, T. (2000) Contour feather moult of ruffs *Philomachus pugnax* during northward migration, with notes on homology of nuptial plumages in scolopacid waders. *Ibis* 142, 289–296.
- K**
- Karell, P., Kontiainen, P., Pietiäinen, H., Siitari, H. & Brommer, J. E. (2008) Maternal effects on offspring IGs and egg size in relation to natural and experimentally improved food supply. *Functional Ecology*, 22, 682–690.
- Kelly, J.F. (2000) Stable isotopes of carbon and nitrogen in the study of avian and mammalian trophic ecology. *Canadian Journal of Zoology*, 78, 1–27.
- Kempenaers, B., Peters, A. & Foerster, K. (2008) Sources of individual variation in plasma testosterone levels. *Philosophical Transactions of the Royal Society London Series B, Biological Sciences* 363, 1711–1723.
- Ketterson, E. D. & Nolan, V. (1999) Adaptation, exaptation, and constraint: a hormonal perspective. *American Naturalist* 154, S4–S25.
- Kis, J., Liker, A. & Székely, T. (2000) Nest defence by lapwings: observations on natural behaviour and an experiment. *Ardea* 88, 155–163.
- Klaassen, M., Lindström, Å., Møltofte, H. & Piersma, T. (2001) Arctic waders are not capital breeders. *Nature* 413, 794.
- Kleijn, D., Berendse, F., Smit, R. & Gilissen, N. (2001) Agri-environment schemes do not effectively protect biodiversity in Dutch agricultural landscapes. *Nature* 413, 723–725.
- Kleijn, D. & van Zuijlen, G. J. C. (2004) The conservation effects of meadow bird agreements on farmland in Zeeland, The Netherlands, in the period 1989–1995. *Biological Conservation* 117, 443–451.
- Kleijn, D., Schekkerman, H., Dimmer, W. J., van Kats, R. J. M., Melman, T. C. P. & Teunissen, W. A. (in press) Adverse effects of agricultural intensification and climate change on breeding habitat quality of Black-tailed godwits *Limosa l. limosa* in the Netherlands. *Ibis*, in press.
- Klomp, H. (1951) Over de achteruitgang van de Kieviet, *Vanellus vanellus* in Nederland en gegevens over het legmechanisme en het eiproductievermogen. *Ardea* 39, 143–182.
- Klomp, H. (1970) The determination of clutch-size in birds. A review. *Ardea* 58, 1–124.
- Kokko, H. (1999) Competition for early arrival in migratory birds. *Journal of Animal Ecology* 68, 940–950.
- Kruk, M., Noordervliet, M. A. W. & ter Keurs, W. J. (1996) Hatching dates of waders and mowing dates in intensively exploited grassland areas in different years. - *Biological Conservation* 77, 213–218.
- Kruk, M., Noordervliet, M. A. W. & ter Keurs, W. J. (1997) Survival of black-tailed godwit Chicks *Limosa limosa* in intensively exploited Grassland Areas in The Netherlands. *Biological Conservation* 80, 127–133.
- Kuijper, D.P.J., Wymenga, E., van der Kamp, J., Tanger, D. (2006) Wintering areas and spring migration of the black-tailed godwit (*Limosa limosa*); bottlenecks and protection along the migration route. A&W-rapport 820, Altenburg en Wymenga ecologisch onderzoek, Veenwouden.
- L**
- Lack, D. (1950) Family-size in titmice of the genus *Parus*. *Evolution* 4, 279–290.
- Lains, P. & Sousa, P. (1998) Estatística e produção agrícola em Portugal, 1846–1915. *Análise Social* 33, 935–968.
- Lange, G. (1968) Über Nahrung, Nahrungsaufnahme und Verdauungstrakt mitteleuropäischer Limikolen. *Beiträge zur Vogelkunde* 13, 225–334.
- Larsen, T., Sordahl, T.A., Byrkjedahl, I. (1996) Factors related to aggressive nest protection behaviour: a comparative study of Holarctic waders. *Biological Journal of the Linnean Society* 58, 409–439.

- Lebreton, J. D., Burnham, K. P., Clobert, J. & Anderson, D. R. (1992) Modeling survival and testing biological hypotheses using marked animals: a unified approach with case studies. *Ecological Monographs* 62, 67–118.
- Lee, P. L. M. & Griffiths, R. (2003) Sexing errors among museum skins of a sexually monomorphic bird, the moorhen *Gallinula chloropus*. *Ibis* 145, 695–698.
- Lee, P. L. M., Brain, P. F., Forman, D.W., Bradbury, R. B. & Griffiths, R. (2002) Sex and death: CHD1Z associated with high mortality in moorhens. *Proceedings of the Royal Society London Series B* 56, 2548–2553.
- Lessells, C. M. & Boag, P. T. (1987) Unrepeatable repeatabilities: a common mistake. *Auk* 104, 116–121.
- Liebezeit, J. R., Smith, P. A., Lancot, R. B., Schekkerman, H., Tulp, I., Kendall, S. J., Tracy, D. M., Rodrigues, R. J., Melfote, H., Robinson, J. A., Gratto-Trevor, C., MacCaffery, B. J., Morse, J., & Zack, S. W. (2007) Assessing the development of shorebird eggs using the flotation method: species-specific and generalized regression models. *The Condor* 109, 32–48.
- Lind, H. (1961) Studies of the behavior of the black-tailed godwit (*Limosa limosa*). Munksgaard, Copenhagen.
- Lindström, A., Visser, G. H. & Daan, S. (1993) The energetic cost of feather synthesis is proportional to basal metabolic rate. *Physiological Zoology* 66, 490–510.
- Lourenço, P. M. & Piersma, T. (2008a) Stopover ecology of black-tailed godwits *Limosa limosa* in Portuguese rice fields: A guide on where to feed in winter. *Bird Study* 55, 194–202.
- Lourenço, P. M. & Piersma, T. (2008b) Changes in the non-breeding distribution of continental black-tailed godwits *Limosa limosa limosa* over 50 years: a synthesis of surveys. *Wader Study Group Bulletin* 115, 91–97.
- Loyau, A., Saint Jalme, M., Mauget, R. & Sorci, G. (2007) Male sexual attractiveness affects the investment of maternal resources into the eggs in peafowl (*Pavo cristatus*). *Behavioral Ecology and Sociobiology* 61, 1043–1052.
- M**
- MacDonald, M. A. & Bolton, M. (2008) Predation of Lapwing *Vanellus vanellus* nests on lowland wet grassland in England and Wales: effects of nest density, habitat and predator abundance. *Journal for Ornithology*, 149, 555–563
- MacDougall-Shackleton, S. A. & Hahn, T. P. (2007) Adaptation and evolution of photoperiod response systems in birds. *Journal of Ornithology* 148, Suppl. 2, 219–224.
- Mace, G. M. & Pelletier, F. (2007) Commentary on Ricklefs & Cadena (2007): Reproductive investment and lifespan. *Ecology Letters* 10, 872–874.
- Marra, P. P., Hobson, K. A. & Holmes, R.T. (1998) Linking winter and summer events in a migratory bird by using stable-carbon isotopes. *Science* 282, 1884–1886.
- Mayr, E. (1982) The growth of biological thought: diversity, evolution, and inheritance. Harvard University Press, Harvard.
- McGraw K. J. & Hill G. E. (2000) Differential effects of endoparasitism on the expression of carotenoid- and melanin-based ornamental coloration. *Proceedings of the Royal Society London Series B* 267, 1525–1531.
- McGraw K. J., Mackillop E. A., Dale J. & Hauber M.E. (2002) Different colors reveal different information: How nutritional stress affects the expression of melanin- and structurally based ornamental plumage. *The Journal of Experimental Biology* 205, 3747–3755.
- McGraw, K. J., Dale, J. & Mackillop, E. A. (2003) Social environment during molt and the expression of melanin-based plumage pigmentation in male house sparrows (*Passer domesticus*). *Behavioral Ecology and Sociobiology* 53, 116–122.
- McGraw, K. J. (2008) An update on the honesty of melanin-based color signals in birds. *Pigment Cell Melanoma Research* 21, 133–138.
- Meijer, T. & Drent, R. (1999) Re-examination of the capital and income dichotomy in breeding birds. *Ibis* 141, 399–414.
- Miller-Rushing, A. J., Lloyd-Evans, T. L., Primack, R. B. & Satzing, P. (2008) Bird migration times, climate change, and changing population sizes. *Global Change Biology* 14, 1959–1972.
- Mizutani, H., Fukuda, M., Kabaya, Y. & Wada, E. (1990) Carbon isotope ratio of feathers reveals feeding behavior of cormorants. *Auk* 107, 400–437.

Monaghan, P., Nager, R. G. & Houston, D. C. (1998) The price of eggs: increased investment in egg production reduces the offspring rearing capacity of parents. *Proceedings of the Royal Society London Series B* 265, 1731–1735.

N

Nadeau, N. J., Burke, T. & Mundy, N. I. (2007) Evolution of an avian pigmentation gene correlates with a measure of sexual selection. *Proceedings of the Royal Society London Series B* 274, 1807–1813.

Nager, R. (2006) The challenges of making eggs. *Ardea* 94, 323–346.

Nager, R. G., Monaghan, P., & Houston, D. C. (2001) The cost of egg production: increased egg production reduces future fitness in gulls. *Journal of Avian Biology* 32, 2, 159–166.

Nakagawa, S., Ockendon, N., Gillespie, D. O. S., Hatchwell, B. J. & Burke, T. (2007) Assessing the function of house sparrows' bib size using a flexible meta-analysis method. *Behavioral Ecology* 18, 831–840.

Niecke, M., Rothlaender, S. & Roulin, A. (2003) Why do melanin ornaments signal individual quality? Insights from metal element analysis of barn owl feathers. *Oecologia* 137, 153–158.

Nolet, B.A. & Klaassen, M. (2005) Time and energy constraints in demanding phases of the annual cycle: an example of time limitation in refuelling migratory swans. *Oikos* 111, 302–310.

Nooker, J. K., Dunn, P. O. & Whittingham, L. A. (2005) Effects of food abundance, weather and female condition on reproduction in tree swallows (*Tachycineta bicolor*). *The Auk* 122, 1255–1238.

Norris, K., Marra, P. P., Kyser, T. K., Sherry, T. W. & Ratcliffe, L. M. (2003): Tropical Winter Habitat limits Reproductive Success on the temperate Breeding Grounds in a migratory Bird. - *Proceedings of the Royal Society London Series B: Biological Science* 271, 59–64.

O

Oring, L. W. & Lank, D. B. (1982) Sexual selection, arrival times, philopatry and site fidelity in the polyandrous spotted sandpiper. *Behavioral Ecology and Sociobiology* 10, 185–191.

Orzack, S. H. & Tuljapurkar, S. (2001) Reproductive effort in variable environments, or environmental variation is for the Birds. *Ecology* 82, 2659–2665.

Owen-Ashley, N. T., Hasselquist, D. & Wingfield, J. C. (2004) Androgens and the immunocompetence handicap hypothesis. Unraveling direct and indirect pathways of immunosuppression in song sparrows. *American Naturalist* 164, 490–505.

Owens I. P. F., Dixon A. & Burke T. (1995) Strategic paternity assurance in the sex-role reversed Eurasian dotterel (*Charadrius morinellus*): behavioral and genetic evidence. *Behavioral Ecology* 6, 14–21.

P

Pain, D. J., Green, R. E., Giessing, B., Kozulin, A., Poluda, A., Ottosson, U., Flade, M., and Hilton, G. M. (2004) Using stable isotopes to investigate migratory connectivity of the globally threatened aquatic warbler *Acrocephalus paludicola*. *Oecologia* 138, 168–174.

Parejo, D., Oro, D. & Danchin, E. (2006) Testing habitat copying in breeding habitat selection in a species adapted to variable environments. *Ibis* 148, 146–154.

Pärt, T. & Doligez, B. (2003) Gathering public information for habitat selection: prospecting birds cue on parental activity. *Proceedings of the Royal Society London Series B: Biological Science* 270, 1809–1813.

Pärt, T. (2001) The effects of territory quality on age-dependent reproductive performance in the Northern wheatear, *Oenanthe oenanthe*. *Animal Behavior* 62, 379–388.

Pearce-Higgins, J. W. & Yalden, D. W. (2004) Habitat selection, diet, arthropod availability and growth of a moorland wader: the ecology of European golden plover *Pluvialis apricaria* chicks. *Ibis* 146, 335–346.

Pearce-Higgins, J. W., Dennis, P., Whittingham, M. J. & Yalden, D. W. (2009) Impacts of climate on prey abundance account for fluctuations in a population of a northern wader at the southern edge of its range. *Global Change Biology*. DOI: 10.1111/j.1365-2486.2009.01883.x

Perrins, C. M. (1970) The timing of birds' breeding seasons. *Ibis* 112, 242–255.

Phillips, R. A. & Furness, R. W. (1998) Polymorphisms, mating preferences and sexual selection in the arctic skua. *Journal of Zoology* 245, 245–252.

- Piersma, T. (2006) Using the power of comparison to explain habitat use and migration strategies of waders worldwide. *Journal of Ornithology* 147 (Suppl.), 5.
- Piersma, T. & Drent, J. (2003) Phenotypic flexibility and the evolution of organismal design. *Trends in Ecology and Evolution* 18, 228–233.
- Piersma, T. & Jukema, J. (1993) Red breasts as honest signals of migratory quality in a long-distance migrant, the bar-tailed godwit. *Condor* 95, 163–177.
- Piersma, T. (1986) Breeding Waders in Europe: a Review of Population Size Estimates and a Biography of Information Sources. - Wader Study Group Bulletin 48 (Suppl.), 1–116.
- Piersma, T., Mendes, L., Hennekens, J., Rattarison, S., Groenewold, S. & Jukema, J. (2001) Breeding plumage honestly signals likelihood of tapeworm infestation in females of a long-distance migrating shorebird, the bar-tailed godwit. *Zoology* 104, 41–48.
- Piersma, T., van Gils, J., & Wiersma, P. (1996) Family Scolopacidae (sandpipers, snipes and phalaropes). In J. del Hoyo, A. Elliott, & J. Sargatal (Eds.), *Handbook of the Birds of the World*, Vol. 3. Hoatzin to Auks (pp. 444–533). Lynx Edicions, Barcelona.
- Pinheiro, J. C. & Bates, D. M. (2000) *Mixed-effects Models in S and S-PLUS*. Springer, New York.
- Pomiankowski, A. (1988) The evolution of female mate preferences for male genetic quality. *Oxford Surveys in Evolutionary Biology* 5, 136–184.
- Price, T., Kirkpatrick, M. & Arnold, S. (1988) Directional Selection and the Evolution of Breeding Date in Birds. *Science* 240, 798–799.
- Prota, G. (1992) *Melanin and Melanogenesis*. Academic Press, New York.
- Przybylo, R., Sheldon, B. C. & Merilä, J. (2000) Climatic effect on breeding and morphology: evidence for phenotypic plasticity. *Journal of Animal Ecology* 69, 395–403.
- Q**
- Quinn, G. P. & Keough, M. J. (2002) *Experimental Design and Data Analysis for Biologists*. Cambridge University Press, Cambridge.
- R**
- R Development Core Team (2008) *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL: <https://www.R-project.org>.
- Ratcliffe, N., Furness, R. & Hamer, K. (1998) The interactive effects of age and food supply on the breeding ecology of great skuas. *Journal of Animal Ecology* 67, 853–862.
- Rendon, M.A., Green, A.J., Aguilera, E. & Almaraz, P. (2008) Status, distribution and long-term changes in the waterbird community wintering in Doñana, south-west Spain. *Biological Conservation* 141, 1371–1388.
- Reneerkens, J. & Korsten P. (2004) Plumage reflectance is not affected by preen wax composition in red knots *Calidris canutus*. *Journal of Avian Biology* 35:405–409.
- Ricklefs, R. E. & Cadena, C. D. (2007) Lifespan is unrelated to investment in reproduction in populations of mammals and birds in captivity. *Ecology Letters* 10, 867–875.
- Ricklefs, R. E. (1977) On the evolution of reproductive strategies in birds: reproductive effort. *American Naturalist* 111, 453–478.
- Rjabow, W. F. & Mosalowa, N. I. (1967) Besonderheiten der Ernährung von Brachvogel und Uferschnepfe in Nordkasachstan. *Ornitologija* 8, 211–220.
- Robertson, B. C. & Gemmel, N. J. (2006) PCR-based sexing in conservation biology: Wrong answers from an accurate methodology? *Conservation Genetics* 7, 267–271.
- Robinson, R. A., Crick, H. Q. P., Learmonth, J. A., Maclean, I. M. D., Thomas, C. D., Bairlein, F., Forchhammer, M. C., Francis, C. M., Gill, J. A., Godley, B. J., Harwood, J., Hays, G. C., Huntley, B., Hutson, A. M., Pierce, G. J., Rehfish, M. M, Sims, D. W., Begoña Santos, M., Sparks, T. H., Stroud, D. A. & Visser, M. E. (2008) Travelling through a warming world: climate change and migratory species. *Endangered Species Research* DOI: 10.3354/esr00095.
- Romanoff, A. L. & Romanoff, A. J. (1949) *The avian egg*. Wiley, New York.
- Roodbergen, M., Klok, C. & Schekkerman, H. (2008) The ongoing decline of the breeding population of black-tailed godwits *Limosa l. limosa* in The Netherlands is not explained by changes in adult survival. *Ardea* 96, 207–218.
- Roodbergen, M. & Klok, C. (2008) Timing of breeding and reproductive output in two black-tailed godwit *Limosa limosa* populations in The Netherlands. *Ardea* 96, 219–232.

- Roulin, A., Almasi, B., Rossi-Pedrucci, A., Ducrest, A.-L., Wakamatsu, K., Miksik, I., Blount, J. D., Jenni-Eiermann, S. & Jenni, L. (2008) Corticosterone mediates the condition-dependent component of melanin-based coloration. *Animal Behavior* 75, 1351–1358.
- Roy, S. & Gilbert, W. (2006) The evolution of spliceosomal introns: patterns, puzzles and progress. *Nature Reviews Genetics* 7, 211–221.
- Rubenstein, D. R. & Hauber, M. E. (2008) Dynamic feedback between phenotype and physiology in sexually selected traits. *Trends in Ecology and Evolution* 23, 655–658.
- Rubenstein, D. R. & Hobson, K. A. (2004) From birds to butterflies: animal movement patterns and stable isotopes. *Trends in Ecology & Evolution* 19, 256.
- Rubenstein, D. R., Chamberlain, C. P., Holmes, R. T., Ayres, M. P., Waldbauer, J. R., Graves, G. R. & Tuross, N.C. (2002) Linking breeding and wintering ranges of a migratory songbird using stable isotopes. *Science* 295, 1062–1065.
- Ryan, M. J. (1997) Sexual selection and mate choice. In: *Behavioral ecology - an evolutionary approach* (Davies, N. B. & Krebs, J. R., eds). Blackwell Science Ltd., Malden.
- S**
- Sæther, B. E. (1990) Age-specific variation in reproductive performance of birds. In: *Current ornithology* 7 (ed. Power, D. M.). Plenum Press, New York.
- Sætre, G., Borge, T., Lindroos, K., Haavie, J., Sheldon, B. C., Primmer, C. & Syvänen, A.-C. (2003) Sex chromosome evolution and speciation in *Ficedula* flycatchers. *Proceedings of the Royal Society London Series B* 270, 53–59.
- Sætre, G., Moum, T., Bures, S., Král, M., Adamjan, M. & Moreno, J. (1997) A sexually selected character displacement in flycatchers reinforces premating isolation. *Nature* 387, 589–592.
- Safran, R. J., Adelman, J. S., McGraw, K. M. & Hau, M. (2008) Sexual signal exaggeration affects physiological state in male barn swallows. *Current Biology* 18, R461–R462.
- Sánchez-Guzmán, J. M., Moran, R., Masero, J. A., Corbacho, C., Costillo, E., Villegas, A. & Santiago-Quesada, F. (2007) Identifying new buffer areas for conserving waterbirds in the Mediterranean basin: The importance of the rice fields in Extremadura, Spain. *Biodiversity and Conservation* 16, 3333–3344.
- Sanchez-Lafuente, A. M. (2004) Trade-off between clutch size and egg mass, and their effects on hatchability and chick mass in semi-precocial purple swamphen. *Ardeola* 51, 319–330.
- Sandercock B. K. (1998) Assortative mating and sexual size dimorphism in Western and Semipalmated Sandpipers. *Auk* 115, 786–791.
- Sandercock, B.K. (2001) What is the relative importance of sexual selection and ecological processes in the evolution of sexual size dimorphism in monogamous shorebirds? *Wader Study Group Bulletin* 96, 64–70.
- Santiago-Quesada, F., Masero, J. A., Albano, N., Villegas, A. & Sánchez-Guzmán, J. M. (2009) Sex differences in digestive traits in sexually size-dimorphic birds: Insights from an assimilation efficiency experiment on black-tailed godwit. *Comparative Biochemistry and Physiology Part A* 152, 565–568.
- Schekkerman, H. & Müskens, G. (2000). Do Black-tailed Godwits *Limosa limosa* breeding in agricultural grasslands produce sufficient young for a stable population? *Limosa* 73, 121–134.
- Schekkerman, H. & Beintema, A. J. (2007) Abundance of invertebrates and foraging success of black-tailed godwit *Limosa limosa* chicks in relation to agricultural grassland management. *Ardea* 95, 39–54.
- Schekkerman, H. & Visser, H. (2001) Prefledging energy requirements in shorebirds: energetic implications of self-feeding precocial development. *Auk* 188, 944–957.
- Schekkerman, H. (2008) Precocial problems. PhD Thesis, University of Groningen, The Netherlands.
- Schekkerman, H., Teunissen, W. & Oosterveld, E. (2008) The effect of mosaic management on the demography of black-tailed godwit *Limosa limosa* on farmland. *Journal of Applied Ecology*, 45, 1067–1075.
- Schekkerman, H., Teunissen, W. & Oosterveld, E. (2009) Mortality of black-tailed godwit *Limosa limosa* and northern lapwing *Vanellus vanellus* chicks in wet grasslands: influence of predation and agriculture. *Journal of Ornithology* 150, 133–145.

- Schroeder, J., Groen, N. & Both, C. (2005) Grutto's leggen kleinere eieren later in het seizoen. - Twirre - natuur in Fryslân 16, 219.
- Schroeder, J., Lourenço, P., van der Felde, M., Hooijmeijer, J., Both, C., & Piersma, T. (2008a) Sexual dimorphism in plumage and size in black-tailed godwits (*Limosa limosa limosa*). *Ardea* 96, 25–37.
- Schroeder, J., Heckroth, M. & Clemens, T. (2008b) Against the trend: increasing numbers of breeding Northern Lapwings *Vanellus vanellus* and black-tailed godwits *Limosa limosa limosa* on a German wadden sea island. *Bird Study* 15, 100–107.
- Schroeder, J., Lourenço, P. M., Hooijmeijer, J. C. E. W., Both, C., & Piersma, T. (2006) Assortative mating by plumage traits in the black-tailed godwit (*Limosa l. limosa*). Poster contribution at the 24th International Ornithological Congress 2006, Hamburg, Germany.
- Schroeder, J., Lourenço, P. M., Hooijmeijer, J. C. E. W., Both, C. & Piersma, T. (2009) A possible case of contemporary selection leading to a decrease in sexual plumage dimorphism in a grassland-breeding shorebird. *Behavioral Ecology* 20, 797–807.
- Senar, J.C. & Quesada, J. (2006) Absolute and relative signals: a comparison between melanin- and carotenoid-based patches. *Behavior* 143, 589–595.
- Siiikamäki, P. (1998) Limitation of reproductive success by food availability and breeding time in pied flycatchers. *Ecology* 79, 1789–1796.
- Simons, A. (2009) Fluctuating natural selection accounts for the evolution of diversification between hedging. *Proceedings of the Royal Society London Series B*. Online early, DOI: 10.1098/rspb.2008.1920
- Soma, K. K. (2006) Testosterone and aggression: Berthold, birds and beyond. *Journal of Neuroendocrinology* 18, 543–551.
- Sordahl, T. A. (1990) There is a risk of avian mobbing behavior. *Wilson Bulletin* 102, 349–352.
- Staav, R. & Fransson T. (2008) EURING list of longevity records for European birds URL: http://www.euring.org/data_and_codes/longevity.htm
- Stearns, S. C. (1992) *The Evolution of Life Histories*. Oxford University Press, Oxford.
- Sterbetz, I. (1962) Ecological problems of the *Limosa limosa* L. after the changes of landscape in Hungary. Józsa András Muz. Évkönyve, Nyiregyháza 4–5, 211–218.
- Stokes, D. G. & Perry, R. P. (1995) The DNA-binding and chromatin-localization properties of CHD1. *Molecular and Cellular Biology* 15, 2745–2753.
- Summers, R. W. & Underhill, L. G. (1991) The relationship between body size and time of breeding in Icelandic Redshanks *Tringa t. robusta*. *Ibis* 133, 134–139.
- Székely, T., Freckleton, R. P. & Reynolds J. D. (2004) Sexual selection explains Rensch's rule of size dimorphism in shorebirds. *Proceedings of the National Academy of Sciences USA* 101, 12224–12227.
- T**
- Teunissen, W., Schekkerman, H. & Willems, F. (2005) Predatie bij weidevogels, Report No. 2005/11, Beek-Ubbergen: SOVON Vogelonderzoek Nederland.
- Teunissen, W., Schekkerman, H., Willems, F., Majoor, F. (2008) Identifying predators of eggs and chicks of Lapwing *Vanellus vanellus* and black-tailed godwit *Limosa limosa* in the Netherlands and the importance of predation on wader reproductive output. *Ibis* 150, 74–85.
- Teunissen, W.A. & Soldaat, L.L. (2005) Indexen en trends van een aantal weidevogelsoorten uit het Weidevogelmeetnet. Periode 1990–2004. Weidevogelindexen 1990–2004, SOVON-informatie 2005/13. SOVON Vogelonderzoek Nederland.
- Thijssen, J. P. (1904) *Het vogeljaar: handleiding tot het leren kennen der meest voorkomende nederlandse vogels*, Versluys, Amsterdam.
- Thomas, D. W., Blondel, J., Perret, P., Lambrechts, M. M. & Speakman, J. R. (2001) Energetic and fitness costs of mismatching resource supply and demand in seasonally breeding birds. *Science* 291, 2598–2600.
- Thompson, D. R., Furness, R. W., & Lewis, S. A. (1995) Diets and long-term changes in $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values in northern fulmars *Fulmarus glacialis*. *Marine Ecology Progress Series* 125, 3.
- Thorup, O. (2006) Breeding waders in Europe 2000. *International Wader Studies* 14, 58–59.
- Timmerman, A., Bos, D., Ouwehand, J. & de Goede, R. (2006) Long-term effects of fertilization regime on earthworm abundance in a semi-natural grassland area. *Pedobiologia* 50, 427–432.

- Toral, G., Figuerola, J. & Negro, J. (2008) Multiple ways to become red: Pigment identification in red feathers using spectrometry. *Comparative Biochemistry and Physiology. Part B. Biochemistry & Molecular Biology* 150, 147–152.
- Townsend, C. R., Harper, J. L. & Begon, M. E. (2006) *Ecology. From Individuals to Ecosystems*. Blackwell Publishing, Malden.
- Trivers, R.L. (1972) Parental investment and sexual selection. In: *Sexual Selection and the Descent of Man* (ed. Cambell B.). Aldine-Atherton, Chicago.
- Tryjanowski, P. & Sparks, T. (2001) Is the detection of the first arrival date of migrating birds influenced by population size? A case study of the red-backed shrike *Lanius collurio*. *International Journal of Biometeorology* 45, 217–219.
- Tulp, I. & Schekkerman, H. (2008) Has prey availability for arctic birds advanced with climate change? Hindcasting the abundance of tundra arthropods using weather and seasonal variation. *Arctic* 61, 48–60.
- V**
- van de Pol, M., Bakker, T., Saaltink, D. & Verhulst, S. (2006) Rearing conditions determine offspring survival independent of egg quality: A cross-foster experiment with oystercatchers *Haematopus ostralegus*. *Ibis* 148, 203–210.
- van den Brink, V., Schroeder, J., Both, C., Lourenço, P. M., Hooijmeijer, J. C. E. W. & Piersma, T. (2008) Space use by black-tailed godwits *Limosa limosa limosa* during settlement at a previous or a new nest location. *Bird Study* 55, 188–193.
- van der Meer, J. & Piersma, T. (1994) Physiologically inspired regression models for estimating and predicting nutrient stores and their composition in birds. *Physiological Zoology* 67, 305–329.
- van Noordwijk, A. J. & Müller, C. B. (1994) On adaptive plasticity in reproductive traits, illustrated with laydate in the great tit and colony inception in a bumble bee. In: *Animal Societies; Individuals, Interactions and Organisation*. (eds. P. J. Jarman & A. Rossiter) Kyoto University Press, Kyoto.
- van Noordwijk, A. J. & Thomson, D. L. (2008) Survival rates of black-tailed godwits *Limosa limosa* breeding in The Netherlands estimated from ring recoveries. *Ardea* 96, 47–57.
- van Paassen, A. G., Veldman, D. H. & Beintema, A. J. (1984) A simple device for determination of incubation stages in eggs. *Wildfowl* 35, 173–178.
- Verhulst, J., Kleijn, D. & Berendse, F. (2007) Direct and indirect effects of the most widely implemented Dutch agri-environment schemes on breeding waders. *Journal of Applied Ecology* 44, 70–80.
- Visser, M. E. & Both, C. (2005) Shifts in phenology due to global climate change: the need for a yardstick. *Proceedings of the Royal Society of London, Series B* 272, 2561–2569.
- Visser, M. E., van Noordwijk, A. J., Tinbergen, J. M. & Lessells, C. M. (1998) Warmer springs lead to mistimed reproduction in great tits (*Parus major*). *Proceedings of the Royal Society of London, Series B* 265, 1867–1870.
- Visser, M.E. & Lessells, C.M. (2001) The costs of egg production and incubation in great tits (*Parus major*). *Proceedings of the Royal Society London Series B* 268 1271–1277.
- W**
- Wallander, J., Blomqvist, D. & Lifjeld, J. T. (2001) Genetic and social monogamy - does it occur without mate guarding in the ringed plover? *Ethology* 107, 561–572.
- Walsh, P. S., Metzger, D.A., & Higuchi, R. (1991) Chelex-100 as medium for simple extraction of DNA for PCR-based typing from forensic material. *Biotechniques* 10, 506.
- Ward, M. P. (2005) Habitat selection by dispersing Yellow-headed Blackbirds: evidence of prospecting and the use of public information. *Oecologia* 145, 650–657
- Watkins, D. (1993) A national plan for shorebird conservation in Australia. Canberra: Australasian Wader Studies Group, Royal Australasian Ornithologists Union and World Wide Fund for Nature. P 33.
- Webster, M.S., Marra, P.P., Haig, S.M., Bensch, S., & Holmes, R.T. (2002) Links between worlds: unraveling migratory connectivity. *Trends in Ecology & Evolution*, 17, 76.
- Weimerskirch, H., Zimmermann, L. & Prince, P. A. (2001) Influence of environmental variability on breeding effort in a long-lived seabird, the yellow-nosed albatross. *Behavioral Ecology*, 12, 22–30.

- Weladji, R. B., Loison, A., Gaillard, J. M., Holand, O., Mysterud, A., Yoccoz, N. G., Nieminen, M. & Stenseth, N. C. (2008) Heterogeneity in individual quality overrides costs of reproduction in female reindeer. *Oecologia* 156, 237–247.
- West-Eberhard, M. J. (1989) Phenotypic plasticity and the origin of diversity. *Annual Review of Ecology and Systematics* 20, 249–278.
- White, G. C. & Burnham, K. P. (1999) Program MARK: survival estimation from populations of marked animals. *Bird Study*, 46, 120–139.
- Whittingham, M. J. & Evans, K. L. (2004) The effects of habitat structure on predation risk of birds in agricultural landscapes. *Ibis* 146, 210–220.
- Wiens, J. J. (2001) Widespread loss of sexually selected traits: How the peacock lost its spots. *Trends in Ecology and Evolution* 16, 517–523.
- Wikelski, M., Lynn, S., Breuner, C., Wingfield, J. C. & Kenagy, G. J. (1999) Energy metabolism, testosterone and corticosterone in white-crowned sparrows. *Journal of Comparative Physiology A* 185, 463–470.
- Wilbur H. M. & Rudolf, V. H. W. (2006) Life-history evolution in uncertain environments: Bet hedging in time. *American Naturalist* 168, 398–411.
- Williams, T. D. (2005) Mechanisms underlying the costs of egg production. *BioScience* 55, 39–48.
- Y**
- Yohannes, E., Hobson, K. A., Pearson, D., & Wassenaar, L. I. (2006) Stable isotope analyses of feathers help identify autumn stopover sites of three long-distance migrants in northeastern Africa. *Journal of Avian Biology* 36, 235.
- Z**
- Zahavi, A. (1975) Mate selection - a selection for a handicap. *Journal of Theoretical Biology* 53, 205–214.
- Zwarts, L., Bijlsma, R., van der Kamp, J. & Wymenga, E. (2009) Living on the edge. Wetlands and birds in a changing Sahel. KNNV publishing, Zeist.

Summaries

Fryske Gearfetting – Frisian summary

YN DIT WURK sykje en besprekke wy fitnesskorrelaasjes yn de skries (*Limosa l. limosa*). Myn kollega's en ik bestudearje patroanen dy't ynteressant wêze kinne foar de measte yntegrative evolusjonêre ekologen. Wy bediskusjearje korrelaasjes en harren suggestive krêft fan hoe't seleksje útwurkje kin en ek útwurkje kinnen hie op de skries. De wrâld dêr't wy yn libje, feroaret oan ien tried wei, yn ús tiid flugger as ea. It gefolch dêrfan is dat natuerlike fermiddens om yn te libjen yn minder as inkelde tsientallen jierren yngripend feroarje kinne. Minsken hawwe hast alle ekologyske sônes en typen fan habitat dêr't oare soarten yn libje beynfloede en feroare. Der binne frijwat soarten dy't har der op talein hawwe om te libjen yn in lânskip dat troch minsken makke is en om dêr ek foar in neiteam te soargjen. In lyts part fan dy soarten bestiet út de greidefûgels, mei ûnder har de skries. Feroaringen fan in lânskip kinne ferskillen feroarsaakje yn seleksjedruk. Foar de skries jildt dat sawol de bried- as de net-briedomkriten de lêste twa ieuwen bot feroare binne.

Yn Nederlân briede skriezen fan âlds yn sompen en feangebieten. Yn de earste helte fan de foarige ieu hawwe in protte fan dy gebieten in agraryske bestimming krigen. Skriezen pasten, lykas oare greidefûgels, harsels oan en begûnen te brieden op kultivearre lân. Yn earste opslach wie dat foardielich en in sterke populaasjegroei foarme it begjin fan 'de gouden ieu' fan de greidefûgels – fûgels dy't greidelân brûkten dat troch minsken makke wie. Greidefûgels wienen al gau populêr by boeren, natuerleafhawwers en fûgelders. Se hearden foargoed ta de Nederlânske greidegebieten. De skries waard, as 'kening fan de greidefûgels', it populêrst. Om 1960 hinne koenen jo de skries foaral yn greidelân fine en hast net mear yn sompen en feangebieten.

Nei de útfining fan de keunstdong begûn in twadde feroaring fan it lânskip. Yntinsivearring fan de lânbou, benammen yn de foarm fan wetterdrainazje, hege stikstofjeften en nije, fluch groeiende plantesoarten, hawwe it mooglik makke om mear as ien kear yn it jier te rispen, mei in hieltiten hegere opbringst. Dat hat earder meanen mooglik makke op it no yntinsyf behearde agrarysk greidelân, dat boppedat in folle lytser ferskaat oan planten en ynsekten hie. Dy gong fan saken wie net geunstich foar greidefûgels: ferlies fan it lechsel troch it brûken fan masinen, net genôch fretten foar de pykjes, in taname fan predaasje troch te min dekking nei it meanen en habitatferlies yn it algemien hawwe in grutte ôfname fan it tal briedende greidefûgels feroarsake. Sûnt de ein fan de jierren santich is de populaasje oan skriezen mei 5% yn it jier ôfnommen en ek al is de skries as kening fan de greidefûgels noch altiten in like wolkomme gast, der is gjin reden om oan te nimmen dat dat proses fluch ophâlde sil.

De skries hat ek te meitsjen krigen mei in dramatyske feroaring bûten it briedgebiet, te witten op de plakken dêr't se yn de iere maitiid byinoar komme. Sûnt 1920 binne in soad oarspronklik wiete gebieten op it Ibearyske Skiereilân útdroege en tagelyk is earder droech lân omfoarme ta rysplantaazjes. Fan âlds kamen skriezen yn natuerlike swietwettergebieten byinoar, mar yn sterk kontrast dêrmei sykje se tsjintwurd-

dich, as se net fleane, meastentiids har fretten byinoar yn de foarm fan weigriemde of ferbarnde ryskerlen op grutte rysfjilden.

Gearfetsjend: skriezen hawwe in protte feroaringen yn har libbensgebieten meimakke. Dat jildt foar it hiele geografyske ferspriedingsgebiet. Dêrom is it ynteressant om fitnesskorrelaasjes te ûndersykjen en har ferbân mei feroarjende seleksjiedruk.

Yn **haadstik twa** beskriuwe wy it gedrach fan skriezen nei har oankomst oan de ein fan de winter yn de briedgebieten yn Nederlân. Wy litte sjen dat de fûgels weromkomme op har briedplak fan it ôfrûne jier en dat se foar in perioade fan inkelde dagen oant in pear wiken tichtby dy lokaasje bliuwe. Fûgels dy't letter wer op har âld nestplak briede, bliuwe dêr yn 'e regel inkelde wiken, oant de lisdatum, wylst fûgels dy't úteinlings op in oar plak begjinne te brieden, har stadichoan fierder fan it plak fan oankomst bejouwe. Sok gedrach suggerearret dat se in soarte fan in ferkenningsgedrach fertoane. Dat is in nij ynsjoch, want earder waard tocht dat de kar fan in (nij) nêstplak tastân kaam op grûn fan de ûnderfiningen dy't de skries it jiers dêrfoar op syn briedplak opdien hie. Us ûndersyk lit lykwols sjen dat it beslút om dochs earne oars te brieden, foarôfgongen troch it ferkennen fan oare lokaasjes, ek yn it nije jier nommen wurde kin. De resultaten litte ek sjen dat de territoaria betreklik koart nei de oankomst ynnommen wurde en dat de fûgels oant de lisdatum hiel faak yn de direkte omkriten fan it nêst tahâlde. Dat ynsjoch hat konsekwinssjes foar bygelyks de rol fan de eksakte romtlike parameters dy't de kwaliteit fan de habitat bepale. Ta beslút yntrodusearje wy de kearn fan ús ûndersyksgebiet, de Warkumerwaard.

Yn **boks B** litte wy sjen dat betiid lizzen in foardiel wêze kin foar skriezen, sels wannear't se briede yn in natuerreservaat mei in rezjym fan let meanen.

Yn **haadstik trije** is te lêzen dat it neidiel fan let brieden de lêste trije desennia tanommen is, nei alle gedachten troch de ynfloed fan de minske. Ferrassend genôch kinne wy gjin feroaring fan lisdatum fine. Hjoeddeistige skriezen ynvestearje lykwols mear as se 30 jier ferlyn dienen yn betide lechsels, benammen yn jierren mei in foarôfgeande waarme winter. Wy litte ek sjen dat it folume fan it aai, it gewicht fan de pyk en de timing fan it brieden de oerlibbingskânsen fan it pykje bepale en dus kritysk binne foar rekrutearingsrasjo's.

Yn **haadstik fjouwer** kwantifisearje wy seksueel dimorfisme fan it fearrekleed en lichemsôfmjittingen. Wy komme ta de konklúzje dat sommige eigenskippen fan it fearrekleed ûnder seksuele seleksje stean. Dêrneist herevaluearje wy molekulêre seksing metoaden en stelle in oplossing út foar in faak foarkommende molekulêre seksing flater. Wy yntrodusearje hjir foar it earst dat fariaasje op it CHD1-Z allel kofariearje kin mei fitnesskorrelaasjes yn de skries, wat suggerearret dat der in genetyske basis wêze kin foar ferskate fenotypes fan it fearrekleed.

Yn **haadstik fiif** brûke wy de hjirfoar definiearre skoares oangeande it fearrekleed en ûndersykje oft se korrelearre binne oan reproduktif súkses yn ús hjoeddeistige briedpopulaasje fan skriezen. Wy ha útfûn dat se yndied korrelearre binne, mar op in hiel ûnferwachte manier: bleke mantsjes binne better. Dat hat ús ta de hypoteze brocht dat bleke mantsje by seleksje yn it foardiel wêze moatte. En it docht ek werklik bliken dat bleke mantsjes fan it nominale ras yn de lêste 164 jier bleker wurden binne, wat

der ta bydroegen hat dat it seksuele dimorfisme ôfnommen is. Tsjintwurdich binne mantsjes dy't der útsjogge as wyfkes súksesfoller as kleurige mantsjes. Wy suggerearje dat it minder stride hoege om territoaria in ôfname fan seleksjedruk foar kleurige mantsjes feroarsake hat en dat minder fel kleurde mantsjes dêrtroch mooglik in foardiel krigen hawwe.

Yn **haadstik seis** dogge wy in soartgelikense analyze as yn haadstik fiif, mar no foar skriezen fan it Yslânske ûndersoarte, fan wa't de skiednis oars is. Yn dat soarte giet de populaasjegrutte op it stuit omheech en de kompetysje om nêstplakken op briedgebieten fan in hege kwaliteit is nei alle gedachten sterk. Hypotetysk sjoen soe dat in positive relaasje jaan moatte tusken de ornamintaasje fan it fearrekleed fan de mantsjes en it briedsúkses. Wy demonstrearje dat mear ornamintearre mantsjes yndied in pear foarmje mei wyfkes dy't mear ynvestearje yn reproduksje as by mantsjes mei minder ornamintaasje.

Yn **haadstik 7** litte wy sjen dat it in foardiel wêze kin foar skriezen om hiel ticht op inoar te brieden en wy spekulêrje oer de mooglikheid dat in fel kleurd fearrekleed skriezen behinderje kin om sa hiel deun byinoar te brieden.

Yn **haadstik 8** herhelje wy it ûndersyk fan haadstik fjouwer en teste in grutter part fan de populaasje foar kofariaasje fan de intron-fariaasje fan *CHD1-Z* mei fitnesskorrelaasjes. It docht bliken dat it fearrekleed fan skriezen yndied in genetyske basis hat. Wy demonstrearje dat dy marker allinnich oanwêzich is by skriezen mei in hege kwaliteit dy't briede yn gebieten mei ek in hege kwaliteit. Fierder is der bewiis foar populaasjestruktuer yn relaasje mei de kwaliteit fan it briedgebiet, omdat fûgels mei in genetyske marker dy't hege kwaliteit oanjout, allinnich mar fûn wurde yn briedgebieten mei in hege kwaliteit, mar nea der bûten. Wy fine ek dat it fêststelde polymorfisme gjin nije mutaasje is, omdat dy al oanwêzich wie yn in skries dy't dearekke yn 1929 en einige yn it Deensk nasjonaal natuerhistoarysk museum yn Kopenhagen.

Yn **boks C** beskriuwe wy de resultaten fan in ekspearimint op skriezen dy't ûnder de foarjierstrek fêsthâlden waarden. Skriezen dy't mei rys fuorre waarden, namen minder yn gewicht ta as skriezen dy't larven fan miggen foarset krigen, mar se ûntwikkelen mear kleur.

Yn **haadstik njoggen** besykje wy om ferskate foerazjear- en rêststrategyen bleat te lizzen. Wy keppelje isotoop-sinjatueren fan briedfearren dy't oanmakke binne op de rêstgebieten yn de maitiid, oan fitnesskorrelaasjes. Wy ha in relaasje fûn tusken de oankomst fan wyfkes yn it briedgebiet, it lichemsgewicht en de isotoop-sinjatuer, en ek fan aafolume en isotoop-sinjatuer, mar wy kinne gjin dúdlike útspraak dwaan oer de oanwêzigens fan in keppeling mei in bepaalde lokaasje, of in bepaald soarte fan iten, foaral net omdat de isotoop-sinjatueren fan ferskate itenssoarten fan ferskate lokaasjes net dúdlik út inoar te hâlden binne.

Yn **haadstik tien** besykje ik ús befiningen ta in gehiel te yntegrearjen. Ik presintearje foarriedige resultaten fan genetyske kofariaasje fan de ornamintaasje fan it fearrekleed, feroarsake troch melanine, mei microsatellyt-fariaasje yn in bepaald gen (*POMC*). Dat gen is ek keppele oan oare eigenskippen, lykas agressyf gedrach, taname fan lichemsgewicht en hormoankonsintraasjes. Ik spekulêrje derop dat dy keppeling

in ferklearing jaan kin foar de ôfname yn seksueel fearrekleed dimorfisme as de ornamintaasje fan it fearrekleed kofariearret mei it nivo fan agressiviteit fan de drager en syn fermogen om te brieden yn in gebiet mei in hege of lege tichtens. Dat soe ek in ferklearing wêze kinne foar de op it each tsjinstridige befiningen út boks C.

Wat de life history-eigenskippen oanbelanget, suggerearje ik dat skriezen har gedrage sa as de teory foarspelt foar langlibjende fûgels: se maksimalisearje har libbensfitness troch maksimalisaasje fan it oerlibjen as folwoeksene, en net troch de jierlikse reprodúktive produksje. Yn in min jier sille skriezen nei alle gedachten ôfsjen fan brieden en har reprodúksje útstelle oant in, better, takomstich jier. De hjoeddeistige, jierlikse fluktuaasjes binne lykwols direksjoneel en net stogastysk; it meanen krijt earder syn beslach en ek feroaringen fan it klimaat geane mar troch. Yn sa'n gefal kin de foarsichtige strategy min útpakke foar fûgels dy't in ynvestearring yn hjoeddeistige produksje ynruilje foar takomstige produksje, omdat dy bettere takomst miskien nea komt. Dat betsjut dat de reprodúksje net heech genôch is om de stjertesifers kompensearje te kinnen en dat soe in ferklearring wêze kinne foar de sterke efterútgong yn populaasje dy't de Nederlânske skriezen mominteel sjen litte.

Yn it besûnder it lêste foarbyld toant dat dit wurk ek tige nijsgjirrich wêze kin foar in tapaste ynfalshoeke. In mear detaillearre ynsjoch yn fitness- korrelaasjes en seleksjiedruk kin kennis opsmite dy't ús yn steat stelt om it ôfnimmen fan de populaasje tsjin te gean. Om de maatregels te beskriuwen dy't dêrfoar needsaaklik binne, haw ik de demografyske gegevens dy't publisearre binne yn de wittenskiplike literatuer, brûkt om de takomst fan de skries yn Nederlân te foarspellen. De útkomst fan dat model jou spitigernôch net folle moed. Yn it bêste gefal en ûnder de bêste betingsten (hege oerlibbing fan de jongen en hege reprodúksje), sil de briedpopulaasje fan de skriezen yn Nederlân miskien yn 2030 likegoed al ûnder de 10 000 briedpearen dûke.

Om de situaasje fan de skries yn Nederlân te ferbetterjen, kinne wy mikke op of ferbettering fan oerlibbingskânsen of fan reprodúksje. Oerlibbing is dreech te feroarjen sjoen de al tige hege jierlikse oerlibbingskânsen fan folwoeksen skriezen. Dêrom woe ik graach foarspelle mei hofolle oft de reprodúksje tanimme moat om de efterútgong fan de populaasje ta stilstân te bringen. Dan docht bliken dat ûnder it bêst mooglike senario, wat wierskynlik te optimistysk tocht is, it reprodúktif súkses ferhege wurde moat nei op syn minst alle jierren 0,85 pykje per briedpear yn Nederlân. De oplossing om de efterútgong ta stilstân te bringen liket yn teory ienfâldich, mar, sa as sa faak, sil de praktyk minder maklik wêze. Der sil mei de eigners fan de hoeken greidelân ûnderhannelen wurde moatte oer har ekonomyske ferwachtingen. Myn konklúzje is dat it kearen fan it efterútgean fan de skriezestân yn Nederlân in ambisjeus en hertferwaarmjend projekt is, mar ûnder de hjoeddeistige kondysjes gjin realistysk doel foarmet. Yn it ljocht fan de hjoeddeistige agraryske ekonomy en polityk falt it te betwiveljen oft wy wol bysteat wêze sille om de maatregels te nimmen dy't needsaaklik binne om de efterútgong fan de skries yn Nederlân tsjin te hâlden of op syn minst stadiger ferrinne te litten. As wy werklik feroaringen wolle, moatte wy de ekonomyske konsekwinsjes dêrfan akseptearje en fluch en flink optrede, foardat it te let is.

Deutsche Zusammenfassung – German Summary

IN DER VORLIEGENDEN ARBEIT haben wir meine Koautoren und ich individuelle Fitness Korrelate in Uferschnepfen (*Limosa limosa*) gesucht, gefunden und diskutiert. Unsere Ergebnisse sind potentiell interessant für Biologen der Evolutionsökologien, gleichzeitig hoffe ich aber, dass unsere Ergebnisse auch im Naturschutz Anwendung Interesse weckenfinden.

Das vorherrschende Thema meiner Arbeit ist in welchem Masse Fitness Korrelate Aufschluss geben können über historische, rezente und sich verändernde Selektionsdrücke, ist das vorherrschende Thema meiner Arbeit. Unsere Umwelt erfährt ständige Veränderungen natürlichen und anthropogenen Ursprungs. Seit der Evolution Erfindung der Landwirtschaft durch den Menschen hat die Rate der Umweltveränderungen zugenommen und im letzten Jahrhundert noch drastisch an Geschwindigkeit und Umfang zugelegt. Wir Menschen haben die meisten, wenn nicht alle, Ökosysteme der Erde massiv und nachhaltig beeinflusst und damit auch die Umwelt aller anderen lebenden Organismen. Solche starken Veränderungen in der Umwelt haben logischerweise Konsequenzen für die Selektionsdrücke die Organismen erfahren und hat. Im Laufe der Zeit hat dies zu Artensterben in bisher ungekannten Ausmassen einer großen Aussterbewelle von Arten geführt.

Nichtsdestotrotz hat eine ganze Reihe von haben einige Arten sich angepasst und lebt leben nun mehr oder weniger erfolgreich in einer anthropogen modifizierten oder oft sogar kreierten erschaffenen Umwelt. Die Wiesenvögel, eine Gruppe von Vogelarten, die landwirtschaftlich genutzte (Feucht)Wiesen bevölkert, gehört zu diesen Kulturfolgern. Dazu gehört auch Ebenso die Uferschnepfe, ein Watvogel der in Wiesen der temperaten Zone Eurasiens brütet und in Feuchtgebieten (meistens Reisfeldern) der Subtropen und Tropen überwintert. Ein Großteil der Uferschnepfenpopulation von Westeuropa brütet in den Niederlanden. Dort, wie auch in den Überwinterungs- und Zuggebieten auf der Iberischen Halbinsel und in Westafrika haben während des letzten Jahrhunderts einschneidende Veränderungen stattgefunden, die zweifelsohne Selektionsdrücke und damit die Fitnesslandschaft für Uferschnepfen verändert haben. Uferschnepfen haben ursprünglich in Niedermooren und Marschen gebrütet. Während der ersten Hälfte des letzten Jahrhunderts jedoch wurden die meisten, beinahe alle, dieser natürlichen Gebiete in landwirtschaftlich nutzbare Flächen umgewandelt. Die Uferschnepfen, ebenso wie andere Wiesenvogelarten, haben erwiesen sich als moderne Kulturfolger erwiesen, pasten sich angepasst an und verlegten die Brutgebiete in Heuwiesen verlegt. Die Bemistung dieser Flächen bewirkte eine Zunahme der Abundanz der Bodenfauna, welche. Diese wiederum ist Hauptbestandteil der Nahrung für Uferschnepfen und anderer Wiesenvögel ist. Von daher war diese Anpassung anfänglich von großem Vorteil und hat leitete ein goldenes Zeitalter der Wiesenvögel eingeleitet: eine Gruppe von häufig vorkommenden Vogelarten, die als Kulturfolger landwirtschaftliche Flächen als Bruthabitat nutzten und deren

Populationsgröße während dieser Zeit deutlich zugenommen hat. Demgegenüber gab es gegen Ab 1960 gab es so gut wie keine Brutvorkommen mehr in Niedermooren. Aufgrund Sicher auch aufgrund ihrer sehr auffälligen Präsenz im Frühjahr und Sommer auf anthropogen genutzten Flächen sind Uferschnepfe, Kiebitz (*Vanellus vanellus*) und Rotschenkel (*Tringa totanus*) als integrativer Bestandteil untrennbar verbunden mit der (Feucht-) Wiesenlandschaft der Norddeutschen Tiefebene und der Grasslandschaft der Niederlande. Wiesenvögel sind gleichermaßen beliebt unter Bauern, Naturliebhabern und Ornithologen, besonders in den Niederlanden, wo die Uferschnepfe als „König der Wiesenvögel“ die Rangliste anführt.

In der Mitte des letzten Jahrhunderts jedoch haben synthetische Mineraldünger, mit der zunehmenden Produktion derer in industriellem Maßstab, ihren Siegeszug in der Landwirtschaft gehalten angetreten und das Bild der Landschaft abermals verändert sich abermals. Die Intensivierung der Landwirtschaft, das Trockenlegen von Feuchtwiesen, hohe Nitritwerte und neue, schnell wachsende Grassarten ermöglichten dem modernen Bauern mehrere Mahdtermine pro Jahr, statt zuvor nur einen. Dies hatte zur Folge, dass auf intensiv bearbeiteten Wiesen, mit Zunahme des Ernteprofits der Ernte, die erste Mahd im Jahr sukzessive früher stattfindet und dass die Artenvielfalt von Insekten und Pflanzen abnimmt. In Folge haben Dies ist den Wiesenvögeln nicht zuträglich. Nest- und Kükenverluste durch mechanische Mahd mit Großfahrzeugen, ein starker Rückgang der Erreichbarkeit und des Vorhandenseins von Nahrung für Küken, eine starke Zunahme von Verlusten durch Prädation durch unzureichende Deckung nach der Mahd, sowie eine Zunahme der Anzahl von Prädatoren und Habitatverluste im allgemeinen haben zu einer rapiden Abnahme der Anzahl brütender Wiesenvögel geführt. Seit Ende der siebziger Jahre hat die Brutpopulation der Uferschnepfe in den Niederlanden jährlich um 5% abgenommen, und trotz der anhaltenden Beliebtheit der Uferschnepfe als König der Wiesen gibt es keinen Grund anzunehmen, dass dieser Rückgang in naher Zukunft nachlassen wird.

Auch in den Überwinterungs- und Zuggebieten hat die Uferschnepfe starke Umweltveränderungen hinnehmen müssen. Seit 1920 verschwanden auf der Iberischen Halbinsel viele natürliche Feuchtgebiete. Auf der anderen Seite wurden auch Ehemals aride Gebiete wurden durch Bewässerungsmaßnahmen in Reisfelder umgewandelt. Uferschnepfen, denen früher die natürlichen Feuchtgebiete als Rastplatz dienten, machen heutzutage beinahe ausschließlich Gebrauch von Reisfeldern und ernähren sich von übrig gebliebenem, bei der Ernte verschüttetem Reis. All diese Veränderungen haben ihren Abdruck in der Naturgeschichte der Uferschnepfe hinterlassen, und diese Arbeit versucht, einige dieser Veränderungen und ihre Wirkung Auswirkungen aufzudecken.

In **Kapitel 2** beschreiben wir das räumliche Verhalten von Uferschnepfen in Relation zu dem Neststandort nach der Ankunft im Brutgebiet in den Niederlanden. Wir zeigen, dass Vögel zuerst an die Stelle des Vorjahresnistplatzes zurückkehren und sich für einige Tage, zum Teil sogar Wochen, in der unmittelbaren Nähe dieser Stelle aufhalten. Uferschnepfen, die später auch wieder denselben Vorjahresnistplatz gebrauchen, bleiben für gewöhnlich in der direkten Umgebung, bis zum Legdatum. Die

(zumeist wenigen) Vögel jedoch, die einen Nestplatzwechsel vornehmen, bewegten sich nach und nach von dem alten Platz weg, hin zu dem neuen Nistplatz. Dieses Verhalten suggeriert, dass im aktuellen Jahr erst der alte Nestplatz inspiziert wird und erst danach potentielle neue Nestplätze inspiziert werden. Dieses Ergebnis ist neu insofern, als dass zuvor angenommen wurde, die Wahl eines (neuen) Nistplatzes wird aufgrund von Erfahrungen am alten Nistplatz des Vogels im Vorjahr getroffen. Unsere Arbeit zeigt jedoch, dass die Entscheidung, wo anders zu brüten, und dass die Erkundung möglicher neuer Nistplätze auch im aktuellen Jahr stattfinden können. Die Ergebnisse zeigen auch, dass Territorien relativ kurz nach der Ankunft im Brutgebiet etabliert werden, und dass die Vögel sich bis zum Legedatum häufig in der direkten Umgebung des Nests aufhalten. Ferner stellen wir in Kapitel 2 den Workumerwaard, das Hauptuntersuchungsgebiet vor.

In **Box B** zeige ich, dass ein frühes Legdatum positiv ist, sogar wenn Uferschnepfen in einem Gebiet brüten, wo in welchem (in Bezug zum Umland) relativ spät gemäht wird. In Kapitel 3 haben wir diese Fragestellung erweitert und wir zeigen, dass der Nachteil von spätem Brüten seit 1976 bis heute stärker geworden ist. Sehr wahrscheinlich geschieht dies aufgrund anthropogener Veränderungen der Umwelt. Wir zeigen, dass Uferschnepfen heutzutage mehr in frühe Gelege investieren, als noch vor 30 Jahren, vor allem in den Jahren, in denen der vorhergehende Winter relativ warm war. Ferner zeigen unsere Ergebnisse, dass Eivolumen, Kükengewicht kurz nach der Schlupf und der Legezeitpunkt die Mortalität der Küken beeinflussen und damit entscheidende Fitness Korrelate sind.

In **Kapitel 4** quantifizieren wir den Sexuellen Dimorphismus in Brutkleid und Körpergröße und -gewicht, und wir folgern, dass einige Brutkleid- Ornamente wahrscheinlich sexuell selektierte Merkmale sind. Zudem evaluieren wir die molekulare Geschlechtsbestimmung der Uferschnepfen in diesem Kapitel neu. Wir schlagen eine Lösung vor, um ein bekanntes, häufig vorkommendes aber genauso häufig übersehenes Problem der molekularen Geschlechtsbestimmung zu beheben. In Kapitel 4 führen wir die Idee ein, dass genetische Variation auf dem CHD1-Z Gen in Uferschnepfen mit Fitness Korrelaten variiert und dass dies möglicherweise Hinweise auf eine genetische Grundlage der Variation im Brutkleid hinweist.

In **Kapitel 5** gebrauchen wir die im vorherigen Kapitel definierten Brutkleid-Merkmale und untersuchen ihre Korrelation mit Reproduktionserfolg und Eivolumen in Uferschnepfenpopulationen im Workumerwaard. Wir finden eine Korrelation, jedoch eine Unerwartete: Weniger bunte Männchen sind mit Weibchen, die größere Eier legen und damit Küken schlüpfen, die höhere Überlebenschancen haben, gepaart, und haben damit einen Vorteil gegenüber auffällig gefärbten Männchen. Dies führt zu der spekulativen Hypothese, dass blasse Männchen selektiert einen Selektionsvorteil haben werden. Um diese zu prüfen, haben wir die Gefiederfärbung von Uferschnepfenbälgen aus Museen untersucht. In der Tat sind Bälge von 1840 bunter als Vögel heutzutage, und die Gefiederfärbung von Männchen, aber nicht die von Weibchen ist in den Jahren weniger auffällig geworden. Heutzutage ähneln Männchen den Weibchen mehr als noch von 164 Jahren, - der sexuelle Dimorphismus hat abgenommen. Wir

spekulieren, dass durch die Intensivierung des Landbaus der Wettbewerb um Territorien zwischen Männchen abgenommen hat, welches die Selektion für bunte Männchen entspannt hat und gleichzeitig weniger bunten Männchen einen Vorteil hattenbietet.

In **Kapitel 6** vertiefen wir unsere Spekulationen, indem wir eine Unterart, die Isländische Uferschnepfe (*Limosa limosa islandica*), untersuchen. In Island brüten Uferschnepfen in, relativ zu den Niederlanden, in natürlichen Gebieten, und es herrscht ein vermutlich starker Wettbewerb um gute Territorien. Deshalb vermuten wir, dass auf Island bunte Männchen besser sind, - entgegengesetzt zu dem Ergebnis, das wir in den Niederlanden gefunden haben. In der Tat können wir zeigen, dass auf Island buntere Männchen mit Weibchen gepaart sind, die größere Eier legen.

In **Kapitel 7** zeigen wir, dass dieser Vorteil möglicherweise über die Nestdichte operiert: Vögel, die weniger bunt sind, brüten in höheren Dichtentheiten als auffällig bunte Vögel. Größere Dichtentheiten führen zu einem höheren Bruterfolg, weil Nester durch mehr Vögel gegen Prädatoren verteidigt werden können.

In **Kapitel 8** wiederholen und erweitern wir die Untersuchung aus Kapitel 4. Wir testen eine größere Anzahl Uferschnepfen aus Brutgebieten unterschiedlicher Qualität auf Kovariation von Fitness Korrelaten mit dem Auftreten des Z* Markers auf dem CHD1-Z Gen. Zusätzlich finden wir diesen Marker nur in Vögeln, die in Brutgebieten hoher Qualität brüten, jedoch nicht in Vögeln aus anderen Gebieten. Außerdem zeigen wir, dass dieser Polymorphismus nicht eine neue Mutation ist, da wir ihn in einem Museumsbalg von 1929 nachweisen konnten.

In **Box C** beschreiben wir die Resultate eines Experiments an Uferschnepfen in Gefangenschaft, während der Vorjahrsrast auf der Iberischen Halbinsel: Uferschnepfen, denen Reis gefüttert wurde, legten weniger an Gewicht zu, mauserten jedoch in ein bunteres Brutkleid als Uferschnepfen, denen Fliegenlarven gefüttert wurden.

In **Kapitel 9** haben wir versucht, mittels einer Analyse von stabilen Isotopen, verschiedene Zugstrategien aufzudecken. Wir haben Isotopsignaturen von Brutfedern, die während der Rast auf der Iberischen Halbinsel produziert wurden, in Relation zu Fitness Korrelaten untersucht. Wir finden Korrelationen zwischen der Ankunft der Weibchen im Brutgebiet, Gewicht der Weibchen, Eivolumen und Isotopensignatur, jedoch können wir über die Art der Diät und den geographischen Standort, wo an denen die Nährstoffe, aus denen die Federn entstanden sind, aufgenommen wurden, keine klare Aussage treffen, da die Isotopensignaturen verschiedener Diäten von verschiedenen Orten nicht deutlich voneinander zu unterscheiden waren.

In **Kapitel 10**, diskutiere ich unsere Ergebnisse und versuche, ein übergreifendes Bild zu erstellen. Ich präsentiere vorläufige Ergebnisse, die zeigen, dass das Brutgefieder, dessen Farbe auf Melanin beruht, in Uferschnepfen mit genetischer Variation auf einem Gen (POMC Gen) variiert, welches wiederum (über pleiotropische Effekte) mit aggressivem Verhalten, Gewichtszunahme und Hormontitern kovariiert. Ich spekuliere, dass ein derartiges Zusammenspiel zwischen Aggressivität und Brutkleidfärbung die Abnahme des sexuellen Dimorphismus (Kapitel 5), die Korrelation von

Dichtheit und Brutkleidfärbung (Kapitel 6) und die widersprüchlichen Ergebnisse aus Box D erklären kann.

Bezüglich der life-history Merkmale schlage ich vor, dass Uferschnepfen sich verhalten, wie wir es von der Theorie erwarten würden. Sie maximieren die Erwachsenenüberlebenswahrscheinlichkeit der Adulten, indem sie in „schlechten“ Jahren weniger oder gar nichts in Reproduktion investieren. Auf diese Weise können langlebige Organismen ihre Fitness maximieren, Fortpflanzung wird nur in guten Jahren riskiert, wo man selber wenige Risiken (qua Überlebenswahrscheinlichkeit) eingehen muss. Das bedeutet, dass in schlechten Jahren quasi auf das nächste, gute Jahr gewettet wird. Wenn jedoch, wie im Falle der Klimaerwärmung, Veränderungen über die Jahre nicht zufällig, sondern gerichtet sind, dann wird die bessere Zukunft nicht kommeneintreten. So eine Strategie führt zu einer nicht ausreichenden Reproduktion, und hat letztlich, wie im Falle der Uferschnepfe, direkte negative Konsequenzen auf für die Populationsdynamik.

Gerade dieses letzte Beispiel zeigt, dass meine Arbeit auch von einer angewandten Seite her interessant ist. Ein besseres, detailliertes Wissen über Fitness Korrelate und Selektionsdrücke ist hilfreich, wenn wir den Populationsrückgang stoppen aufhalten wollen. Um diesen zu quantifizieren, gebrauche ich ein mathematisches Populationsmodell um die demografische Parameter, die notwendig sind, den Populationsrückgang aufzuhalten zu stoppen, zu berechnen. Die Vorhersagen aus diesem Modell sind mehr als enttäuschend. Unter den besten Vorraussetzungen wird die Niederländische Uferschnepfenpopulation bereits 2030 weniger als 10 000 Brutpaare betragen. Um dies zu verhindern, können wir entweder versuchen, die Mortalitätsrate zu verkleinern verringern oder die Brutproduktivität zu erhöhen. Ersteres ist schwierig zu verändern, da erwachsene Uferschnepfen bereits eine sehr lange Lebenserwartung haben. Deshalb will ich mit meinem Modell vorhersagen, um wie viel die jährliche Produktion für eine selbsterhaltende Population erhöht werden müsste. Mein Modell zeigt, dass unter den besten (sehr wahrscheinlich sehr optimistischen) Annahmen, welche sehr wahrscheinlich optimistisch sind, jedes Uferschnepfenbrutpaar in den Niederlanden jedes Jahr mindestens 0.85 flügge Jungtiere produzieren muss. Dies ist ein sehr hoher Wert, vor allem als nationaler (auf die Niederlande bezogener) Durchschnittswert.

Den Populationsrückgang zu stoppen ist klingt schlicht einfach in der Theorie, in der Praxis jedoch wird die Realisation Umsetzung schwierig, da Landbesitzer und Bauern Ansprüche und ökonomische Erwartungen haben, die nur schwierig, wenn überhaupt, damit in Einklang zu bringen sind. Daraus folgt, dass den Populationsrückgang der Uferschnepfe stoppen aufhalten zu wollen, unter den gegebenen Umständen ein hehres, jedoch auch sehr ambitioniertes, wenn nicht sogar fast unrealistisches Ziel ist. Um es notwendigerweise trotzdem zu erreichen, müssen wir nicht nur schnell und entschieden handeln, sondern auch gegebenenfalls unangenehme ökonomische Konsequenzen akzeptieren.

Nederlandse samenvatting – Dutch summary

IN DIT WERK zoeken en bespreken we fitness correlaties in de grutto. (*Limosa l. limosa*). Ik en mijn collega's bestuderen patronen die voor de meeste integratieve evolutionair ecologen interessant kunnen zijn. Onze wereld verandert voortdurend, momenteel met grote snelheid, waardoor leefomgevingen drastisch kunnen veranderen binnen slechts enkele tientallen jaren. De mens heeft vrijwel alle ecologische zones en soorten habitat die door andere organismen op deze wereld bewoond worden beïnvloed en veranderd. Nogal wat soorten hebben zichzelf erop toegelegd om te leven van en zich voort te planten in door mensen gecreëerde landschappen. Een kleine selectie hiervan bestaat uit de weidevogels; onder hen de grutto. Omgevingsveranderingen kunnen verschillen in selectiedruk veroorzaken, en zowel de broed- als de overwinteringsgebieden van grutto's zijn de laatste twee eeuwen sterk veranderd.

In Nederland broedden grutto's oorspronkelijk in moerassen en veengebieden. Gedurende de eerste helft van de laatste eeuw werden veel van deze gebieden omgevormd voor agrarische doeleinden. Grutto's, net als andere weidevogelsoorten pasten zich aan en begonnen te broeden op gecultiveerd land. In eerste instantie was dit voordelig, en een sterke populatiegroei initieerde de 'gouden eeuw' van de weidevogels – vogels die door mensen gemaakt grasland gebruikten. Weidevogels werden al gauw erg populair bij zowel boeren, natuurliefhebbers en vogelaars. Ze raakten onafscheidelijk verbonden met de Nederlandse agrarische gebieden, waar de grutto het meest populair werd als de "Koning der weidevogels". Rond 1960 werden grutto's vooral aangetroffen in grasland en nauwelijks nog in moerassen en veengebieden.

Na de uitvinding van kunstmest trad echter een tweede verandering van het landschap op. Intensivering van de landbouw, in het bijzonder water drainage, hoge stikstofgiften en nieuwe, snelgroeiende plantensoorten, stonden meer dan één oogst, met toenemende opbrengst, per jaar toe. Dit leidde tot vroeger maaien op het nu intensief beheerde agrarische grasland, met een lage planten- en insectendiversiteit. Dit was geen gunstige ontwikkeling voor weidevogels: legselverlies door agrarische mechanische activiteiten, onvoldoende voedselbeschikbaarheid voor kuikens, toegenomen predatie door verminderde dekking na maaien, en algemeen habitatverlies leidden tot een snelle afname van broedende weidevogels. Sinds het einde van de jaren zeventig is de populatie grutto's met 5% per jaar afgenomen, en ondanks de onveranderde populariteit die de grutto als koning der weidevogels heeft is er geen reden om aan te nemen dat dit proces snel zal stoppen.

De grutto ondervond ook een drastische verandering buiten het broedgebied, op de lente-verzamergebieden. Sinds 1920 zijn vele natuurlijke natte gebieden op het Iberisch schiereiland uitgedroogd en tegelijkertijd is voormalig droog land omgevormd tot rijstplantages. Historisch verzamelden grutto's in natuurlijke zoetwatergebieden en in sterk contrast hiermee foerageren ze tegenwoordig tijdens de voorjaars stopover voornamelijk van gemorste en soms verbrande rijstkorrels in grote rijstvelden.

Samenvattend; grutto's hebben veel veranderingen in hun leefgebieden meegeemaakt, over hun gehele geografische verspreidingsgebied. Daarom is het interessant om fitness correlaties en hun relatie met veranderende selectiedruk te bestuderen, en hun relatie met veranderende selectiedruk.

In **hoofdstuk twee** beschrijven we het gedrag van grutto's nadat ze aan het einde van de winter aankomen op hun broedgebieden in Nederland. We laten zien dat de vogels terugkeren naar hun broedplek van het vorige jaar en voor een periode van enkele dagen tot een paar weken in de nabijheid van die locatie verblijven. Vogels die later op hun oude nestplaats broeden blijven voor enkele weken dichtbij, terwijl vogels die uiteindelijk ergens anders gaan broeden, langzaam maar zeker verder weg trekken, wat een soort verkennings gedrag suggereert. Dit is een nieuw inzicht, want eerder werd gedacht dat de meeste vogels de toekomstige kwaliteit inschatten met behulp van reproductief succes van hun broedplaats in het voorgaande broedseizoen, en beslissingen over waar in de toekomst te broeden baseren op deze informatie. We laten tevens zien dat territoria gevormd kort na aankomst gevormd worden en dat vogels het grootste deel van hun tijd doorbrengen in een zeer klein gebied dicht bij hun toekomstige nestplek. Dit heeft consequenties voor, bijvoorbeeld de rol van exacte ruimtelijke verdeling van parameters die habitatkwaliteit bepalen. In dit hoofdstuk introduceren we tevens het kern-studiegebied, de Workumerwaard.

In **box B** laten we zien dat vroeg leggen voordelig kan zijn voor grutto's, zelfs wanneer ze broeden in een natuurreservaat met een regime van laat maaien. In **hoofdstuk drie** laten we zien dat kosten van laat broeden over de laatste drie eeuwen decennia zijn toegenomen, hoogstwaarschijnlijk door menselijke invloed. Verrassend genoeg vinden we geen verandering van legdatum. We laten zien dat grutto's tegenwoordig meer investeren in vroege legsels, in het bijzonder in jaren met een voorgaande warme winter, meer dan ze 30 jaar geleden zouden doen. Tevens tonen we aan dat volume van het ei, kuikengewicht en timing van broeden kuikenoverleving bepalen en dus cruciaal zijn voor recruteringsratio's.

In **hoofdstuk vier** kwantificeren we sexueel dimorfisme van verenkleed en lichaamsafmetingen, en we leiden af dat sommige verenkleed eigenschappen onder sexuele selectie staan. Daarnaast herevalueren we moleculaire sexing methodes en stellen een oplossing voor een veelvoorkomende moleculaire sexing fout. We introduceren hier voor het eerst dat variatie op het CHD1-Z allel kan covariëren met fitness correlaties in de grutto, wat suggereert dat er een genetische basis kan zijn voor verschillende fenotypes van het verenkleed.

In **hoofdstuk vijf** gebruiken we de in het voorgaande gedefinieerde verenkleed scores en onderzoeken of ze gecorreleerd zijn aan reproductief succes in onze tegenwoordige grutto broedpopulatie. We vinden uit dat ze gecorreleerd zijn, maar op een geheel onverwachte manier: blekere mannetjes zijn beter. Dit leidt ons tot de hypothese dat bleke mannetjes in het voordeel moeten zijn bij selectie. En inderdaad blijkt dat mannetjes grutto's van het nominale ras over de laatste 164 jaar bleker geworden zijn, wat ertoe geleid heeft dat het sexuele dimorfisme afgenomen is. Tegenwoordig zijn mannetjes die er meer uitzien als wijfjes succesvoller dan kleurrijke mannetjes. We

suggereren dat verminderde competitie om territoria geleid kan hebben tot een verminderde selectiedruk voor felgekleurde mannetjes en dat dit minder gekleurde mannetjes bevoordeeld kan hebben.

In **hoofdstuk zes** verrichten we een vergelijkbare analyse als in hoofdstuk vijf, echter voor grutto's van de IJslandse ondersoort, die een andere geschiedenis hebben. In deze soort neemt de populatiegrootte momenteel toe en competitie voor nestplaatsen op broedgebieden van hoge kwaliteit is vermoedelijk hoog, wat hypothetisch gezien zou moeten leiden tot een positieve relatie tussen mannelijke verenkleed ornamentatie en broedsucces. We demonstreren dat meer geornamenteerde mannetjes inderdaad gepaard zijn met wijfjes die meer investeren in reproductie dan minder geornamenteerde mannetjes.

In **hoofdstuk zeven** laten we zien dat broeden in hoge dichtheden voordelig kan zijn, en we speculeren we over de mogelijkheid dat een kleurrijk verenkleed grutto's zou kunnen verhinderen om in hoge dichtheden te broeden.

In hoofdstuk acht herhalen we de oefening van hoofdstuk vier en testen een groter deel van de populatie voor covariatie van de intron variatie van CHD1-Z met fitnesscorrelaties. Hieruit blijkt dat grutto verenkleed inderdaad een genetische basis heeft. We demonstreren dat deze marker aanwezig is vogels van hoge kwaliteit die broeden in broedgebieden van hoge kwaliteit. Verder is er bewijs voor populatiestructuur in relatie tot broedgebied kwaliteit, omdat vogels met de hoge kwaliteit genetische marker alleen aangetroffen worden in hoge kwaliteit broedgebied, maar nooit erbuiten. We vinden ook dat dit polymorfisme geen recente mutatie is, aangezien het al aanwezig was in een grutto die stierf in 1929 en eindigde in het Deens nationaal natuurhistorisch museum in Kopenhagen.

In **box C** beschrijven we de resultaten van een experiment op grutto's in gevangenschap tijdens de voorjaarstrek. Grutto's die gevoerd werden met rijst hadden een kleinere gewichtstoename maar ontwikkelden een meer kleurvol broedkleed dan grutto's gevoerd met vliegenlarven.

In **hoofdstuk negen** proberen we verschillende foerageer- en stagingstrategieën te ontrafelen. We koppelen isotoop signaturen van broedveren aangemaakt op de voorjaars staging verzamelgebieden aan fitness correlaties. We vinden een relatie tussen aankomst van wijfjes in het broedgebied, lichaamsgewicht en isotoop signatuur, en ook van eivolume en isotoopsignatuur, maar we kunnen geen duidelijke uitspraak doen over de aanwezigheid van een koppeling met een bepaalde locatie, of een bepaalde soort voedsel, voornamelijk omdat isotoopsignaturen van verschillende voedselsoorten van verschillende locaties niet duidelijk van elkaar te onderscheiden zijn.

In **hoofdstuk tien** probeer ik onze bevindingen te met elkaar te integreren. Ik presenteer voorlopige resultaten van genetische covariatie van door melanine veroorzaakte verenkleed ornamentatie in grutto's met microsatelliet variatie op een bepaald gen (POMC). Dit gen is ook gekoppeld aan andere eigenschappen, zoals agressief gedrag, lichaamsgewichtstoename en hormoon concentraties. Ik speculeer dat deze koppeling de afname in seksueel verenkleed dimorfisme kan verklaren, als verenkleed ornamentatie covarieert met het niveau van agressiviteit van de drager, en zijn

vermogen om in hoge of lage dichtheden te broeden. Dit zou ook als verklaring kunnen dienen voor de ogenschijnlijk tegenstrijdige bevindingen van box C.

Wat betreft life history eigenschappen suggereer ik dat grutto's zich gedragen zoals de theorie voorspelt voor langlevende vogels: ze maximaliseren levensfitness door volwassen overleving te maximaliseren, en niet jaarlijkse reproductieve productie. In een slecht jaar zullen grutto's waarschijnlijk afzien van broeden en reproductie uitstellen tot een beter, toekomstig, jaar. De huidige jaarlijkse fluctuaties zijn echter directioneel en niet stochastisch: maaien vindt steeds vroeger plaats, en ook klimaatverandering gaat steeds verder. In zo'n geval kan deze voorzichtige strategie slecht uitpakken voor vogels die investering in huidige reproductie inruilen voor overleving, omdat die betere toekomst misschien nooit komt. Dit betekent dat reproductie niet hoog genoeg is om voor sterfte te kunnen compenseren, wat de sterke populatieachteruitgang kan veroorzaken die de Nederlandse grutto's momenteel vertonen.

In het bijzonder dit laatste voorbeeld laat zien dat dit werk ook erg interessant kan zijn vanuit een toegepaste invalshoek. Een meer gedetailleerd inzicht in fitness correlaties en selectiedruk kan ons kennis opleveren die ons in staat stelt om de hefbomen te plaatsen die de populatieafname kunnen stoppen. Om de benodigde maatregelen te beschrijven heb ik demografische gegevens, gepubliceerd in wetenschappelijke literatuur, gebruikt om de toekomst van de grutto in Nederland te voorspellen. De uitkomst van dit model is helaas redelijk somber. Onder de beste mogelijke voorwaarden (hoge volwassen overleving en hoge reproductie) zal de Nederlandse broedpopulatie van grutto's mogelijk al in 2030 onder de 10 000 broedparen duiken.

Om de situatie van de grutto in Nederland te verbeteren kunnen we mikken op ofwel verbetering van de overlevingskansen ofwel van de reproductie. Overleving is lastig te veranderen, gezien de al erg hoge jaarlijkse overlevingskans van grutto's. Daarom wilde ik voorspellen hoeveel de reproductie moet verbeteren om de populatie achteruitgang tot stand te brengen. Dan blijkt dat onder het best mogelijke scenario, wat waarschijnlijk redelijk optimistisch is, het reproductieve succes per paar verhoogd moet worden naar ten minste 0,85 jong per grutto paar broedend in Nederland voor elk jaar. De oplossing om de achteruitgang tot staan te brengen lijkt eenvoudig in theorie, maar zoals zo vaak zal de praktijk weerbarstiger zijn. Er zal onderhandeld moeten worden wat betreft de economische verwachtingen van de eigenaren van de graslanden. Mijn conclusie is dat het stoppen van de populatie achteruitgang van de Nederlandse grutto een erg ambitieus en hartverwarmend doel is; echter onder de huidige condities is het geen realistisch doel. Bezien in het licht van de eisen van de huidige agrarische economie en politiek valt het te betwijfelen of we in staat zullen zijn om de maatregelen te nemen die noodzakelijk zijn om de achteruitgang van de grutto in Nederland te stoppen of op zijn minst te vertragen. Als we verandering willen moeten we de economische consequenties aanvaarden en snel en krachtadig optreden, voordat het te laat is.

Acknowledgements

I WOULD LIKE TO THANK EVERYBODY, without whose help, support and input this thesis would not have been possible.

First and foremost I want to thank all volunteers, nature lovers, farmers, birders, members of the Fûgelwacht Warkum and It Fryske Gea who ~~searched~~ found many godwit nests, allowed us access to their land, read many colour rings and contributed information, gossip and cake. Thank you all very, very much for the generosity, trust and friendliness, with which you shared your knowledge, time and data with me. Your support was the most crucial part needed for this work to be completed.

Next, I would like to thank my supervisors, Theunis and Christiaan. You have been great supervisors, supportive, critical yet friendly, available and very helpful at the right times. In many respects you have been the perfect team of supervisors, complementing each other. Next, my thanks go to Jos Hooijmeijer who organized the gazillion things that need to be organized and without whose influence and knowledge, many things we did may not have been possible at all. Further, I want to thank everybody who joined the field work for so many reasons, be it work, study, fun or friendship. Niko Groen also deserves special attention here, because not only did he share his abundant godwit-wisdom, -lore and -experience, and discussed statistics with me, he also provided data for chapters 3 and 6. Thank you for your trust and support, this thesis would be thinner without your data! Many thanks go to Alex Roulin, for being on my reading committee and for commenting on my manuscripts.

All the members of the Animal Ecology Group played an important role, and I want to thank all of them for inspiring lunch meeting talks and helpful comments. I especially want to thank Fardo, Freek, Marcel and Valentijn, - the three Musketeers and D'artagnan! Without you, field work (and other times, too) would have been much less funny and enjoyable! Roos, Pedro, Petra, Jordi, Glenn, Kathie, Maja, Arjen, Robert, Laura and all others who joined field work enthusiastically and the discussion on godwit science with me, thanks a lot for the enjoyable company and input. I want to thank especially Debbie, Yvonne and Maaïke for frequent and resourceful discussion, dinners, academic and personal support, coffee, cake and beer. I want to thank Suus Bakker-Geluk, Joyce Rietfield, Dick Visser and Nelly Eck for helpful and very enjoyable support during the last five years. Jan Komdeur and Maurine Dietz deserve thanks here for being (maybe unknowingly) role models for us, proof that a couple, both working in science, actually can have a family.

The support from the curators of the natural history musea, Kees Rooselaar (Amsterdam), Hein van Grouw (Leiden), Peter Koomen (Leeuwarden), Jon Fjeldså (Kopenhagen), who allowed me access to their collection, was very important. Thank you! A special thanks goes to Pieter Breuker, who not only allowed me to join his class "Fries voor niet-Friestaligen" but also took the time and effort to proof read the Frisian summary, which must have been a tedious task!

I had a great time visiting the department of Conservation Studies of the University of Extremadura (twice), observing and catching “our” godwits preparing for spring migration, conducting experiments on godwits, going bird watching, for a good meal or a beer (or both). Many thanks go to Jose Antonio, who introduced me to the group and to Auxie, Paco, Noe, Pepo and Juanma. I want to especially thank Jose for making it possible for me to come visit together with my infant son, who was allowed to join Spanish daycare and even joined us godwit catching. Thanks a lot for this very special time and opportunity. Paco, thank you very much for all the logistic support! I hope we can continue working together!

Thanks a lot to the Iceland crew, Maja, Valentijn, Paco, Hans, Jordi, Glenn, Dagmar and Dominic. I had an excellent “not-a-vacation” on Iceland and I would not hesitate a heartbeat to repeat it with you all. I also want to thank the people who made it possible for us to go there, discussed grant applications, logistics and who supported us on Iceland, Jenny Gill, Tomas Gunnarsson, Gerrit Gerritson, Niko Groen and the WIWO.

Many thanks go to the friends I made during my time in Groningen, people who where the basis of my social life and whom I already miss dearly now that many of them (including me) left Groningen again. I want to thank especially Debbie, Tomas, Kathie, Valentijn, Saleta, Francisco, Magdalena, Daan, Barbara, Bernd, Alberto, Asia, Harold, and many others. Kathie, many thanks for your friendship and for being my knitting buddy! Fardo, Valentijn and Marcel worked hard to make sure that the Dutch and Frisian summaries are presentable and understandable. Thank you so much for this effort! Valentijn also had a special role as a colleague and friend during fieldwork, discussion, who first-authored Chapter two and who is paronymphing for me. I value our friendship very much, academically as well as personally, and I am sure it will persist. Tomas and Maria allowed me to hold the promotion party at De Eik, which is a very special place for me. Saleta, thank you so much for your friendship, for agreeing to be my paronymph, for the many hours of babysitting and especially for the excellent time we had living together! I dearly miss our legendary dinners with our friends at De Eik, exchanging cooking recipes and discussing the peculiarities of and how to combine best living abroad, pregnancies, field work, parenthood, statistics, babies, politics and, of course, science!

Debbie deserves very special thanks. She was there to help unload our truck when Martin and I moved to Groningen, and she was there to help load it up again for our move to Sheffield, which is symbolic as to that, despite often being physically absent, it felt like she was (and still is) always there for me as my friend. I am grateful that I could be with her for her defense, and I have just heard that she will make an impressively long detour from Seattle, home to Toronto, via (!!) Groningen just to join my defense! Thank you so much for that, I think it’s the longest detour anyone ever made for me! I am convinced that our friendship will last no matter where we live and how often we meet.

And finally, I want to thank my family. My parents, sister, grandmothers and extended family always supported and endured my headstrong ideas on what to do

with my live, whether it meant to come visit me in South-east Asia, to (reluctantly) let me head off to far-away war-ridden countries for field work or to let me realize my own business ideas and other projects, where the benefits were not always apparent to anyone else but me. I think they were all a bit relieved when I started this PhD-position and my life got a bit more stable. They provided endless help with many things, to name two, babysitting and helping me move house for the x-th time to or from yet again another foreign country, Bavaria or a remote island. Thank you very much for all the support, encouragement, and understanding.

When I applied for this PhD-position, it was not even clear whether Martin would come with me to Groningen, or whether it would be me alone. He came with me, being a constant source of steadiness, comfort and encouragement. You are amazing, - I don't think I would have finished my PhD-thesis without you at my side, enabling me to leave for fieldwork, visits to Spain or conferences; be it with or without children, which often meant you stayed at home as an excellent "single parent".

Now, five years after the rainy day when both of us arrived in Groningen, we left again (on a sunny day, actually) not alone, but with two children. This place and time will be special for me not only because I did my PhD here, but because Clara and Jolan were born here. Rather than handicap me as many people often readily assume, having a family enabled me in many ways, teaching me how to be more efficient and productive at work and how to be a mother at home. Clara and Jolan, you made me take a break from my work every day to care for you and marvel about how amazing you are. You are my motivation and my joy, but most of all, you are incredibly wonderful persons. To all my children.

Addresses of Co-authors

Allan J. Baker, Department of Natural History, Royal Ontario Museum, Toronto, ON M5S 2C6, Canada, and Department of Ecology and Evolutionary Biology, University of Toronto, Toronto ON, Canada

Christiaan Both, Animal Ecology Group, Centre for Ecological and Evolutionary Studies (CEES), University of Groningen, P.O. Box 14, 9750 AA Haren, The Netherlands

Francisco Santiago-Quesada, Grupo de Investigacion en Conservacion, Area de Zoologia, Universidad de Extremadura, Avenida de Elvas s/n, 06071 Badajoz, Spain

Gerrit J. Gerritsen, Veerallee 33, 8019 AD, Zwolle, The Netherlands

Hans Schekkerman, Dutch Centre for Avian Migration and Demography, Netherlands Institute of Ecology (NIOO-KNAW), P.O. Box 47, 6700AA Heteren, The Netherlands

Jordi van Gestel, Animal Ecology Group, Centre for Ecological and Evolutionary Studies (CEES), University of Groningen, P.O. Box 14, 9750 AA Haren, The Netherlands

Jos C.E.W. Hooijmeijer, Animal Ecology Group, Centre for Ecological and Evolutionary Studies (CEES), University of Groningen, P.O. Box 14, 9750 AA Haren, The Netherlands

José A Masero, Grupo de Investigacion en Conservacion, Area de Zoologia, Universidad de Extremadura, Avenida de Elvas s/n, 06071 Badajoz, Spain

Juan M Sánchez-Guzmán, Grupo de Investigacion en Conservacion, Area de Zoologia, Universidad de Extremadura, Avenida de Elvas s/n, 06071 Badajoz, Spain

Maja Roodbergen, SOVON Dutch Centre for Field Ornithology, Rijksweg 178, 6573 DG Beek-Ubbergen, The Netherlands

Marcel Klaassen, Department of Plant-Animal Interactions, Netherlands Institute of Ecology, Royal Netherlands Academy of Arts and Sciences (NIOO-KNAW), The Netherlands

Marco van der Velde, Animal Ecology Group, Centre for Ecological and Evolutionary Studies (CEES), University of Groningen, P.O. Box 14, 9750 AA Haren, The Netherlands

Niko M. Groen, Animal Ecology Group, Centre for Ecological and Evolutionary Studies (CEES), University of Groningen, P.O. Box 14, 9750 AA Haren, The Netherlands

Oliver Haddrath, Department of Natural History, Royal Ontario Museum, Toronto, ON M5S 2C6, Canada, and Department of Ecology and Evolutionary Biology, University of Toronto, Toronto ON, Canada

Pedro M. Lourenço, Animal Ecology Group, Centre for Ecological and Evolutionary Studies (CEES), University of Groningen, P.O. Box 14, 9750 AA Haren, The Netherlands

Rosemarie Kentie, Animal Ecology Group, Centre for Ecological and Evolutionary Studies (CEES), University of Groningen, P.O. Box 14, 9750 AA Haren, The Netherlands

Theunis Piersma, Animal Ecology Group, Centre for Ecological and Evolutionary Studies (CEES), University of Groningen, P.O. Box 14, 9750 AA Haren, The Netherlands; Department of Marine Ecology, Royal Netherlands Institute for Sea Research (NIOZ), P.O. Box 59, 1790 AB Den Burg, Texel, The Netherlands

Valentijn van den Brink, Department of Ecology and Evolution, University of Lausanne, CH-1015 Lausanne, Switzerland

