ENVIRONMENTAL INFLUENCES ON MOULT AND MOVEMENT STRATEGIES IN SOUTHERN AFRICAN WATERFOWL

by

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DECLARATION

This thesis reports original research conducted under the auspices of the Percy FitzPatrick Institute, University of Cape Town. All assistance received has been fully acknowledged. This work has not been submitted in any form for a degree at another university.

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To

Sibusisiwe Ndlovu

my mother and my daughter (named after her)

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GENERAL ABSTRACT

Waterfowl annual life history events in north-temperate regions are driven primarily by predictable seasonal variations in temperature. In contrast, the spatio-temporal availability of adequate resources to waterfowl in the semi-arid regions of southern Africa is determined by rainfall which is extremely variable in timing and intensity. I studied the environmental influences on flight-feather moult and movement strategies of southern African waterfowl. Six duck species that are fairly common in most parts of South Africa were selected for the study, namely; Egyptian Geese *Alopochen aegyptiaca*, Spur-winged Geese *Plectropterus gambensis*, South African Shelducks *Tadorna cana*, Yellow-billed Ducks *Anas undulata*, Red-billed Teals *A. erythrorhyncha* and Southern Pochards *Netta erythrophthalma*. I chose two study sites to represent the extremes of environmental conditions in southern African, namely Barberspan (summer-rainfall region) and Strandfontein (temperate winter-rainfall region). I investigated the underlying responses of Afrotropical waterfowl to stochastic varying environmental conditions in southern Africa and the life-history strategies they have evolved to cope with this variability. The study also compares and contrasts the life-history strategies of southern hemisphere waterfowl with those of the northern hemisphere.

Egyptian Geese undergo substantial phenotypic changes during moult, including asynchronous changes and these appear to have evolved to allow moult to be completed as rapidly as possible in a stochastic environment. Spur-winged Geese, South African Shelducks, Yellow-billed Ducks, Red-billed Teals and Southern Pochards also exhibit strategic fluctuations in body mass and pectoral muscle size that are geared towards reducing wing-loading, enabling birds to fly before moult is completed.

Waterfowl in southern Africa (with the exception of South Africa Shelduck), south-west Australia, Europe and North America time their breeding period to coincide with peaks in the

availability of both food and breeding sites. Northern hemisphere species moult when temperatures are warm and before food and aquatic habitats approach their winter minima. By contrast, southern-hemisphere waterfowl delay the onset of moult until the dry season, when food and aquatic habitats are few, opting to moult when both food and aquatic moulting habitats are few.

Egyptian Geese movement patterns were found to be significantly influenced by their geographic location within southern Africa. Telemetry and colour-rings indicated moderately high moult-site fidelity, despite the anthropogenic modification of South Africa’s landscape, particularly the increased number of permanent artificial water bodies which would be expected to lower moult-site fidelity. Moulting birds displayed crepuscular patterns of activity and nocturnal retreats to safety. Barberspan birds undertake long-distance movements which cannot be interpreted as ‘random wanderings’ between adjacent wetlands. By contrast, movements of Strandfontein birds appeared to be random and over short distances, best explained as nomadism.

I conclude that phenotypic flexibility during moult, moulting in the dry season and strong moult-site fidelity to permanent pans, are important evolutionary adaptations that enhance the survival of waterfowl in semi-arid environments. The findings of the fine-scale telemetry over several seasons provided new insights into how Egyptian Geese interact with southern African environments. This study has demonstrated that many northern hemisphere biological paradigms are not applicable to southern African waterfowl species.

CHAPTER 1

General introduction

The long-term persistence of populations depends on their ability to respond appropriately to changing environments (Levins 1968; Frank and Slatkin 1990). Environmental conditions vary over a range of scales in both time and space. The way that birds experience these variations depends on how long they live, and how often and how far they move (Levins 1968; Gabriel *et al*. 2005). Birds living in variable environments face a problem of prediction. The better they can predict and respond to fitness-relevant changes in the environment, the better the chances are that they will survive and reproduce (Cohen 1966; Wingfield *et al*. 1992). One way that birds can overcome fitness trade-offs is to respond to change by actively choosing suitable habitats (Rueffler *et al*. 2005). Another tactic is to use phenotypic plasticity, where a single genotype gives rise to multiple phenotypes suited for different environmental conditions (Piersma and Drent 2003; Gabriel et al. 2005; Donaldson-Matasci *et al*. 2007). Both adaptations can lead to diversity in morphology and behaviour within a species.

Birds have evolved to survive in diverse environments with different spatio-temporal variability in climate (Owen and Black 1990: Baldwin and Lovvorn 1994). The availability of habitat and food in north-temperate and Arctic regions is determined primarily by large and highly predictable seasonal variations in temperature and day length. North-temperate birds have evolved to synchronise their life-history stages with their environment in a variety of ways to exploit this predictability (Wingfield *et al*. 1992; Svensson 1995; Piersma and Drent 2003; Bêty *et al*. 2004). By contrast, environmental variability (both seasonal and annual) in the warmer, semi-arid regions of southern Africa arises primarily from rainfall that varies across the landscape in both its timing and predictability (Tyson and Preston-Whyte 2000).

Because the availability of habitat and food for waterfowl in semi-arid regions is ultimately determined by rainfall events, there is substantial spatio-temporal variability in the availability of suitable habitat and food resources for both reproduction and moult (Siegfried 1974; Petrie 1998). Given this stochasticity, southern African waterfowl may have adapted to use a variety of environmental factors and cues to fine-tune their life-history ‘timetables’ (Herremans 1999; Herrmann *et al*. 2004).

Much of what we know about waterfowl biology comes from the north-temperate latitudes where water is seldom a limiting resource and its availability is seasonally predictable (Baldassarre and Bolen 1994). In areas where resource availability is less predictable, we may expect to find different responses by waterfowl to environmental fluctuations. In this context, southern African waterfowl offer an interesting case study. The semi-arid region of southern Africa as a whole is considered “water scarce” (Harsch 1992) and, in addition to its aridity, the timing of rainfall events can be highly unpredictable (Tyson and Preston-Whyte 2000). Local variations in rainfall present a challenge for waterfowl, particularly ducks and geese (Anatidae) that require water of sufficient depth for foraging, predator avoidance and flight-feather moult. Few studies, however, have addressed the question of how Southern African waterfowl have adapted to such regional conditions.

Moult

In order to identify critical periods in the annual cycle of waterfowl, it is appropriate to focus on events that incur energy demands over and above the normal nutritional requirements for general maintenance (Fox *et al*. 1998). Reproduction, migration and moult are thought to be three of the most energetically demanding life history stages for waterfowl (Walsberg 1983). A significant amount of research has been dedicated to understanding how environmental variables influence the timing of reproduction and migration in waterfowl (Owen and Black

1990; Rohwer 1992). Moult, however, has been largely overlooked, especially in southern African waterfowl (Owen and Black 1990; Ndlovu *et al*. 2010). Most southern African waterfowl (with the exception of Maccoa Duck *Oxyura maccoa* and African Black Duck *Anas sparsa*) undergo one body feather moult annually, followed by one flight-feather moult (Douthwaite 1976; Dean 1978; Milstein 1993;). Waterfowl can replace body feathers gradually at any locality but for the flight-feather moult, when primary feathers are dropped synchronously, birds must seek the safety of deep water bodies where they remain throughout the duration of flightless moult (Salomonsen 1968). This flightless period in southern African waterfowl lasts 4–6 weeks (Dean 1978; Milstein 1993) and the risks of predation and starvation are significant (Geldenhuys 1981). Whether the flightless moult period of waterfowl presents a period of nutritional stress in their annual cycle has been the subject of considerable debate (Murphy 1996). Therefore, flight-feather moult (termed 'flightless moult' from here on) is a potential constraint on the life-histories of southern African waterfowl and may be a key life-history stage around which other life-history components are scheduled.

Movement

Unlike the well-studied phenomenon of long-distance migration, movement patterns of African waterfowl and the environmental factors influencing non-migratory movement have received little attention, yet movement is an integral component of life-history strategies. Movement enables waterfowl to (1) meet their resource requirements in spatially and temporally changing environments; (2) choose suitable habitats to optimize survival, growth and ultimately reproduction; and (3) avoid unsuitable environments. The nature and extent of African waterfowl movement is poorly understood, as is the timing of movement to and from moulting sites: this limits our understanding of the dynamics of African waterfowl populations in time and space. Such knowledge, especially at a fine scale, will also help identify preferred moulting and breeding habitats.

Environmental influences on moult and movement strategies in southern African waterfowl are not well understood. In this thesis I investigate how such strategies relate to environmental variability which in turn influences food and habitat availability. I further compare and contrast the life-history strategies of waterfowl living in a semi-arid region of the southern hemisphere with those occurring in north-temperate environments.

Study sites

Data collection was carried out from March 2007 until November 2010. Two study sites in South Africa were selected; Barberspan Nature Reserve (26º 33´ S, 25º 37´ E) in the North-West Province and the Strandfontein Wastewater Treatment Works (34º 05´ S, 18º 32´ E) in the Western Cape Province.

Barberspan Nature Reserve is a shallow, perennial, alkaline pan connected to the Hart's River in the semi-arid, summer-rainfall region of South Africa. When full, the pan covers an area of about 1700 ha, but water levels fall substantially during late winter, at which time the pan can shrink to 1300 ha. Surrounding natural habitats are mainly gently undulating and flat, comprising short to very short grasslands interspersed with mixed acacia trees (Mucina *et al*. 2006). Woody species are sparse and consist mainly of *Acacia karoo*, *A. erioloba*, *Celtis africana* and *Rhus lancia*. There are also surrounding farmlands used mainly for maize and sunflower cultivation. Large numbers of waterfowl and waders use the pan for foraging, breeding, roosting and moulting (Milstein 1975; Taylor *et al*. 1999). Barberspan is a Ramsar-designated site, this designation being based in part on the site’s waterbird populations.

Strandfontein Wastewater Treatment Works is situated in the winter-rainfall region adjacent to the coast. The site comprises 319 ha of open permanent ponds and canals, some with sandy islands, and 58 ha of terrestrial habitats, much of which is covered with grass. Other vegetation in the area includes exotic *Acacia cyclops* thickets, and patches of *Typha*, *Phragmites* and *Scirpus* spp (Kaletja-Summers *et al*. 2001). The site is hydrologically managed and some ponds have open mudflats that serve at roosting sites for waterfowl and feeding grounds for waders.

Study species

I selected six African duck species that are fairly common and occur in most parts of South Africa, namely: Egyptian Goose *Alopochen aegyptiaca*, Spur-winged Goose *Plectropterus gambensis*, South African Shelduck *Tadorna cana*, Yellow-billed Duck *Anas undulata*, Red-billed Teal *A. erythrorhyncha* and Southern Pochard *Netta erythrophthalma*. I also studied the Egyptian Goose in more detail as a model species to investigate the underlying responses of Afrotropical waterfowl to stochastic environmental conditions in southern Africa and the life-history strategies they have evolved to cope with this variability.

Egyptian Geese are used as a model species because they are one of the most abundant and wide-spread anatids in southern Africa (Hockey *et al*. 2005), have a wide habitat tolerance, and their numbers in South Africa (especially in the Western Cape) are increasing, suggesting adaptability to changing local conditions (Siegfried 1965; Mangnall and Crowe 2002). Reasons for their presence in a diversity of environments, including urban habitats, are not well understood. Possible explanations could be that Egyptian Geese have a diversity of habitat-specific phenotypes, or simply that Egyptian Geese are habitat generalists. In many parts of South Africa the Egyptian Goose is regarded as an agricultural pest that causes significant reductions in grain yields (Maclean 1988), accounting for up to 7% of annual revenue losses incurred by farmers (Mangnall and Crowe 2002). Not only is the species a crop pest, it has also colonised urban habitats, especially in the Western Cape Province of

South Africa, reaching population levels that have required management intervention (Mangnall and Crowe 2002).

Recent studies suggest that wild ducks that have adapted to, and increase in numbers in, anthropogenically altered environments. They also frequently associate with other species (including species other than waterfowl), making them potential vectors of avian influenza (Tracey *et al*. 2004; FAO 2005). Indeed, Egyptian Geese are currently implicated in the spread of low-pathogenic avian influenza virus between ostrich farms and wild duck populations in the Western Cape (Sinclair *et al*. 2006). Therefore, fine-resolution research centred on understanding the movements of Egyptian Geese as they relate to environmental attributes and variation will also contribute to modelling regional disease transmission dynamics (Cumming *et al*. 2011).

Egyptian Geese are long-lived (Underhill *et al*. 1999), are large enough to carry solar-powered Global Positioning System (GPS) transmitters, spend most of the day in direct sunlight, which is ideal for the functioning of solar-powered transmitters, and have a relatively long tarsus that can accommodate large uniquely engraved colour rings.

Thesis outline

In Chapter 2, I analyse physiological changes that occur during moult in Egyptian Geese. I investigate whether, or to what degree, body condition, flight-feather growth, pectoral muscle mass and organ (gizzard, liver and heart) mass of Egyptian Geese show phenotypic flexibility over the short period of flightless moult.

Chapter 3 builds on detailed findings of Egyptian Geese phenotypic flexibility in Chapter 2 and investigates how broadly phenotypic flexibility is evident in other southern African ducks. To test for the existence or extent of interspecific variation in body-mass fluctuations during flight-feather moult, I investigate how the body masses of South African Shelducks, Spur-winged Geese, Yellow-billed Ducks, Southern Pochards and Red-billed Teals change during flight-feather moult. Changes in pectoral muscle size during moult are also investigated in South African Shelducks and Spur-winged Geese.

Changes in environmental conditions act as proximate triggers of important life-history stages such as moult. In Chapter 4, I investigate whether timing of breeding determines the timing of moult or if environmental factors are of overriding importance in determining the timing of moult in southern African waterfowl. I explore how breeding period, temperature and rainfall are related to peak numbers of moulting Spur-winged Geese, Egyptian Geese, South African Shelducks, Yellow-billed Ducks and Redbilled Teals at each study site.

After establishing the extent of environmental influences on moult, I focus on moult-site preference. In Chapter 5, I investigate what proportion of Egyptian Geese moulting at a site return to moult at the same site in the following year and subsequently. I use data from satellite telemetry and uniquely engraved colour rings to quantify moult-site fidelity of Egyptian Geese marked at Barberspan and Strandfontein.

Timing of moult (Chapter 4) and the selection of preferred moult sites (Chapter 5) by a terrestrially foraging and breeding duck mean that Egyptian Geese move regularly between aquatic and terrestrial habitats. Therefore, moult is strongly tied to movement patterns. To understand better the significance of the moult period within the annual cycle, Chapter 6 explores the annual, fine-scale movement patterns of Egyptian Geese. I use satellite telemetry to evaluate daily movement patterns of Egyptian Geese during the flightless moult and flying periods in both the summer and winter rainfall regions of South Africa.

The final chapter is a synthesis of the main results of the study and proposes directions for future research.

Chapter 2 was published in 2010 as: Ndlovu, M, G.S. Cumming, P.A.R Hockey and L.W. Bruinzeel (2010). Phenotypic flexibility of a southern African duck *Alopochen aegyptiaca*: do northern hemisphere paradigms apply? *Journal of Avian Biology* 41:558-564. Contributions of each party to this publication are as follows: I collected, analysed, interpreted the data and wrote the paper. Graeme Cumming and Phil Hockey, as supervisors of the thesis, advised on data collection and commented on each draft of the paper. Leo Bruinzeel and Graeme Cumming contributed to the collection of data.

This thesis was part of a bigger project that studied the movements of waterfowl and the prevalence of avian influenza viruses in southern African (Botswana, Mozambique, South Africa and Zimbabwe) waterbirds. Some of the data that I collected during my PhD study, even though not part of this thesis, complement our understanding of southern African waterfowl and have been used in several, co-authored, peer-reviewed papers (e.g. Cumming and Ndlovu 2011; Cumming *et al*. 2011; Gaidet *et al*. 2012; Cumming *et al*. 2012). I have appended eight co-authored relevant papers, five of which are cited in thesis chapters (Appendix).

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CHAPTER 2

Phenotypic flexibility of a southern African duck during moult: do northern hemisphere paradigms apply?

Abstract

Phenotypic flexibility during moult has never been explored in austral nomadic ducks. I investigated whether the body condition, organ (pectoral muscle, gizzard, liver and heart) mass and flight-feather growth of Egyptian Geese *Alopochen aegyptiaca* in southern Africa show phenotypic flexibility over their 53-day period of flightless moult. Changes in body mass and condition were examined in Egyptian Geese caught at Barberspan and Strandfontein in South Africa. Mean daily change in primary feather length was calculated for moulting geese and birds were dissected for pectoral muscle and internal organ assessment. Mean body mass and condition varied significantly during moult. Body mass and condition started to decrease soon after flight feathers were dropped and continued to do so until the new feathers were at least two-thirds grown, after which birds started to regain body mass and condition. Non-moulting geese had large pectoral muscles, accounting for at least 26% of total body mass. Once moult started, pectoral muscle mass decreased and continued to do so until the flight feathers were at least one-third grown, after which pectoral muscle mass started to increase. The regeneration of pectoral muscles during moult started before birds started to gain overall body mass. Gizzard mass started to increase soon after the onset of moult, reaching a maximum when the flight feathers were two-thirds grown, after which gizzard mass again decreased. Liver mass increased significantly as moult progressed, but heart mass remained constant throughout. Flight feather growth was initially rapid, but slowed towards the completion of moult. These results show that Egyptian Geese exhibit a significant level of phenotypic flexibility when they moult. The observed phenotypic changes are collectively interpreted as an adaptive strategy to minimize the duration of the flightless period. Overall moulting Egyptian Geese in South Africa undergo more substantial phenotypic changes than those reported for ducks in the northern hemisphere.

Introduction

Much of what we know about waterfowl comes from north-temperate latitudes where the availability of water is seasonally predictable and where water is seldom a limiting resource (Baldassarre and Bolen 2006). High predictability in resource availability has allowed north-temperate birds to fine-tune their life histories, dispersal strategies and physiological responses in a variety of ways to exploit this predictability to the fullest (Wingfield *et al*. 1992; Svensson 1995; Piersma and Drent 2003; Bêty *et al*. 2004).

In areas where resource availability is less predictable, we may expect to find different responses by waterfowl to environmental fluctuations. In this context, southern African waterfowl offer an interesting case study. Much of southern Africa is arid and the region as a whole is considered “water scarce” (Harshe 1992). In addition to its aridity, the timing of rainfall events can be highly unpredictable (Tyson and Preston-Whyte 2000). Local variations in rainfall present a challenge for waterfowl, particularly ducks and geese (Anatidae) that need standing water of sufficient depth for foraging, predator avoidance and flight-feather moult.

Ducks undergo a three- to five-week period of flightless moult, during which they replace all of their primary feathers. During this period, grazing ducks and geese generally have to remain close to water and occasionally venture onto land to feed on adjacent, low-quality forage (Halse and Skead 1983; Panek and Majewski 1990). Some of the physiological changes observed in north-temperate ducks and geese during moult have been associated with reductions in mass (Hanson 1962, Folk *et al*. 1966, Hohman *et al*. 1992; Fox and Kahlert 2005). Early studies attributed mass loss to nutritional stress caused by a scarcity of food (Hanson 1962; Folk *et al*. 1966). By contrast, Pehrsson (1987) proposed that mass reduction was an adaptive strategy to reduce wing loading and thereby shorten the flightless period. Recent studies of two north-temperate species, Greylag Goose *Anser anser* (Fox and Kahlert

2005) and Common Scoter *Melanitta nigra* (Fox *et al*. 2008) suggest that the reduction during moult in the mass of body organs associated with flight is an evolutionary adaptation consistent with a trade-off between the importance of their function and cost of their maintenance. It is also interesting to note that most studies of waterfowl (e.g. Mottled Duck *Anas fulvigula* [Fox *et al*. 2008]; Male Mallards *Anas platyrhynchos* [Young and Boag 1982]) report reductions in pectoral muscle size and yet no concomitant decrease in overall body mass as moult progresses, contradicting the reduced wing-loading hypothesis.

If the water body in which a duck is living dries down during the moult period, mortality is likely. It follows that over evolutionary time frames there must have been strong selective pressures for southern African ducks to (1) select permanent (and/or deeper) water bodies for moulting, even if food availability is low; and (2) minimize the duration of the flightless phase of moult.

The extent of phenotypic flexibility that exists in nomadic, south-temperate ducks during moult has never been explored. Indeed, there are few studies globally that address the issue of phenotypic flexibility of moulting waterfowl. Black-necked Grebes *Podiceps nigricollis* have small pectoral muscles during their flightless moult, but during the two weeks prior to departure from the moulting grounds their pectoral muscles double in size (Gaunt *et al*. 1990). To date, no such pattern has been documented for southern African waterfowl. Douthwaite (1976) and Milstein (1993) both reported a decrease in body mass of moulting Red-billed Teals *Anas erythrorhyncha* and seven captive Egyptian Geese *Alopochen aegyptiaca* respectively, but could not link the change to phenotypic flexibility. Rather, they hypothesised that the mass decrease reflected the high energy costs of feather replacement and therefore expected it to continue as moult progressed.

This chapter analyses physiological changes that occur during moult in Egyptian Geese (Anatidae: a true duck, despite its common name). Specifically, I investigate whether or to what degree body condition, flight-feather growth, pectoral muscle mass and organ (gizzard, liver and heart) mass of Egyptian Geese living in variable environments show phenotypic flexibility over the short period of flightless moult. I tested the hypothesis that moulting Egyptian Geese adjust their mass and body condition to minimize the duration of the flightless period (when food availability is low). I expected that: a) moulting ducks would lose weight, body condition, and pectoral muscle mass during moult; and b) shortly before they regained the ability to fly, they would increase their pectoral muscle mass but would depart from their moulting grounds in poor overall body condition (Brown and Saunders 1998).

The study also evaluated two alternative hypotheses. The ‘feather-growth-cost hypothesis’, proposes that flight-feather moult is energetically costly to Egyptian Geese and that this cost will be countered by building up body reserves before commencing moult. Once moult begins, birds are predicted to lose mass (including pectoral muscle mass) and body condition steadily during the moult period (Hanson 1962). The hypothesis does not predict significant change in liver and heart mass, but the gizzard is expected to decrease in mass with limited use (the cost-benefit hypothesis of Piersma and Lindström 1997). The second hypothesis was that remigial moult is not energetically costly to Egyptian Geese. This hypothesis predicts that there will be no significant change in weight, body condition, organ (gizzard, liver and heart) mass or pectoral muscle mass during moult (Ankney 1979; Fox *et al*. 1998)

Materials and methods

Study sites

Adult Egyptian Geese were caught at Barberspan Nature Reserve (26º 33´ S, 25º 37´ E) in the North West Province and Strandfontein Wastewater Treatment Works (34º 05´ S, 18º 32´ E) in the Western Cape Province, South Africa, between March 2007 and February 2008.

Barberspan Nature Reserve is a Ramsar-designated site in a semi-arid, summer rainfall region. It is a perennial pan that is shallow, alkaline and connected to the Hart's River. When full, it covers an area of *ca* 1700 ha, but water levels fall considerably during late winter. Surrounding natural habitats comprise mainly gently undulating and flat, short to very short grassland interspersed with acacia trees. There are also surrounding farmlands used mainly for maize and sunflower cultivation. Large numbers of waterfowl and waders use the pan for foraging, breeding, roosting and moulting. In winter, Egyptian Goose numbers regularly exceed 1000 individuals (Taylor *et al*. 1999).

Strandfontein Wastewater Treatment Works is in a coastal, winter-rainfall area. The site comprises 319 ha of open permanent ponds and canals, some with sandy islands, and 58 ha of terrestrial habitats, much of which is grassy. The woody species found in the area are mainly exotic *Acacia cyclops* (Kaletja-Summers *et al*. 2001). The hydrology of the site is managed and, depending on the management practice of the moment, some ponds have open mudflats that serve at roosting sites for waterfowl and feeding grounds for waders.

Fieldwork

Seven hundred and ten geese were caught during the study, 178 at Barberspan and 532 at Strandfontein, using maize-baited, walk-in traps placed near the water's edge. All geese were banded with metal rings and 410 individuals were also marked with uniquely engraved colour rings. Mass was measured to the nearest gram using a spring balance. Moult, body condition (see below), forewing (to the tip of the longest primary), total head, culmen (to feathering) and tarsus length (from the end of the calcaneal ridge to the base of the last complete scale before the toes diverge) were recorded for all birds. Head, tarsus and culmen lengths were measured with Vernier callipers to the nearest 0.1 mm.

Moult scores were recorded following de Beer *et al*. (2000). For each of the ten primary feathers moult was scored using a six-point scale: 0 = fully grown old (worn) feather, 1 = feather missing or in pin, 2 = feather emerging from sheath up to 33% grown, 3 = new feather 33-67% grown, 4 = new feather from 67% to fully grown with remains of the sheath still visible, 5 = new, fully grown feather with no remaining traces of a sheath. Scores for each feather were summed, giving a total moult score. Because ducks undergo synchronous moult of the remiges, six distinct moult stages (i.e. 0, 10, 20, 30, 40 and 50) are recognizable.

A measure of body condition was derived from a body condition index (BCI) modified from Pehrsson (1987). The BCI was calculated by dividing body mass (g) by the product of two reliable structural measurements, *viz* tarsus and head length. Forewing length was not deemed a reliable structural measure because a) it may vary irrespective of body size (Pehrsson 1987), and b) most of the birds handled in this study were in moult. The BCI thus corrected mass for structural size.

Feather growth of captive birds

Forty adult moulting Egyptian geese (21 in June 2008 and 19 in March 2009) were caught at Barberspan Nature Reserve and housed for 5 days in a walk-in aviary (20 x 3 x 2.5 m). The geese were fed *ad lib.* on poultry grain (a mix of wheat, sorghum and crushed corn) and were free to graze the grass inside the aviary. Clean drinking water was provided daily.

Using these geese, the length (mm) of the right ninth primary flight feather (P9) was measured (from the point where the feather quill emerges out of the forewing to the tip of the feather) on the second day the birds were in captivity and again on the fifth day before they were released. P9 length measurements from 221 non-moulting Egyptian Geese previously captured at Barberspan and Strandfontein during 2007 and 2008 were used to calculate the mean P9 length of non-moulting Egyptian Geese. Assuming constant rates of change in the length of P9 (e.g. van de Wetering and Cooke 2000) the average flightless duration and moult duration were established by dividing the mean P9 length of non-moulting birds by the mean average P9 growth per day of flightless birds and the mean average P9 growth per day of all moulting birds, respectively.

Muscles and organs

Fifteen birds - including three from each of the five moult categories - were collected from different areas around Cape Town. The birds were caught using walk-in traps and then euthanased in carbon monoxide chambers in compliance with Cape Nature permits and University of Cape Town animal ethics regulations. Pectoral muscles (*pectoralis major* and *supracoracoideus*) on both sides of the keel were cut from the sternum and at the underside of the humerus, excised and rinsed in distilled water. The gizzards were removed, emptied, and washed in distilled water. Organs were weighed (wet mass) using a laboratory scale with a 0.01 g precision.

Abdominal and mesenteric fat deposits were extracted and weighed. Subcutaneous fat was scored using Fox *et al*.'s (2008) six-point scale, where 0 = none; 1 = thin layer patchily distributed; 2 = thin layer evenly distributed, no blood vessels; 3 = medium layer, continuous over much of the body, obvious blood supply; 4 = thick layer continuous over body; and 5 = extensive thick deposits throughout, extending down the abdomen and around the neck.

Statistical analysis

A Student's unpaired *t*-test was used to test for significant differences in means of morphometric measurements from the two study sites. The Student's unpaired *t*-test was also used to test for differences in P9 growth rate between flightless and flight-capable moulting Egyptian Geese. I calculated the mean P9 length of non-moulting geese and the mean P9 growth per day of moulting geese. I then fitted a linear regression model to all average P9 increments per day (mm) against initial P9 lengths and estimated the duration of moult and that of the flightless period. A correlation matrix was used to test for interaction terms between body mass, moult, pectoral muscle mass, organ mass and the BCI. Regression models were fitted that best described changes in body mass, pectoral muscles mass, organ mass and body condition as a function of the moult stage. Multivariate analysis of variance (MANOVA) was used to test for the effects of moult stage on weights of pectoral muscle, gizzard, liver and heart. Hypotheses were tested at the 5% level of significance and all analyses were carried out using the package SPSS 17 (SPSS Inc., Chicago IL 2008).

Results

Mean head, culmen and tarsus lengths did not differ between Egyptian Geese at Barberspan and Strandfontein. Non-moulting birds from Strandfontein were slightly, but not significantly heavier than those from Barberspan, with means of 2342 ± 49 g (± C.L) and 2166 ± 55 g respectively. Because there were no significant differences in the metrics of the birds from the two study sites, data were pooled for subsequent analyses.

Two hundred and forty-eight Egyptian Geese with old feathers were caught at Strandfontein. Of these, 62 were moulting body feathers, mainly in the neck and back regions. No body-feather moult was observed on ducks in flight feather moult or on those that had just completed moult. No body moult was observed in all non-moulting Egyptian Geese from Barberspan with both old and new flight feathers.

Mean body mass varied significantly ($F = 45.99$, $P < 0.0001$) over the flightless moult period. Mass started to decrease as soon as the flight feathers were dropped and continued to do so until these flight feathers were at least two-thirds grown, after which mass started to increase (Fig. 1a). Mean BCI followed a similar trend to that of mass (Fig. 1b; $F = 71.17$, $P < 0.0001$).

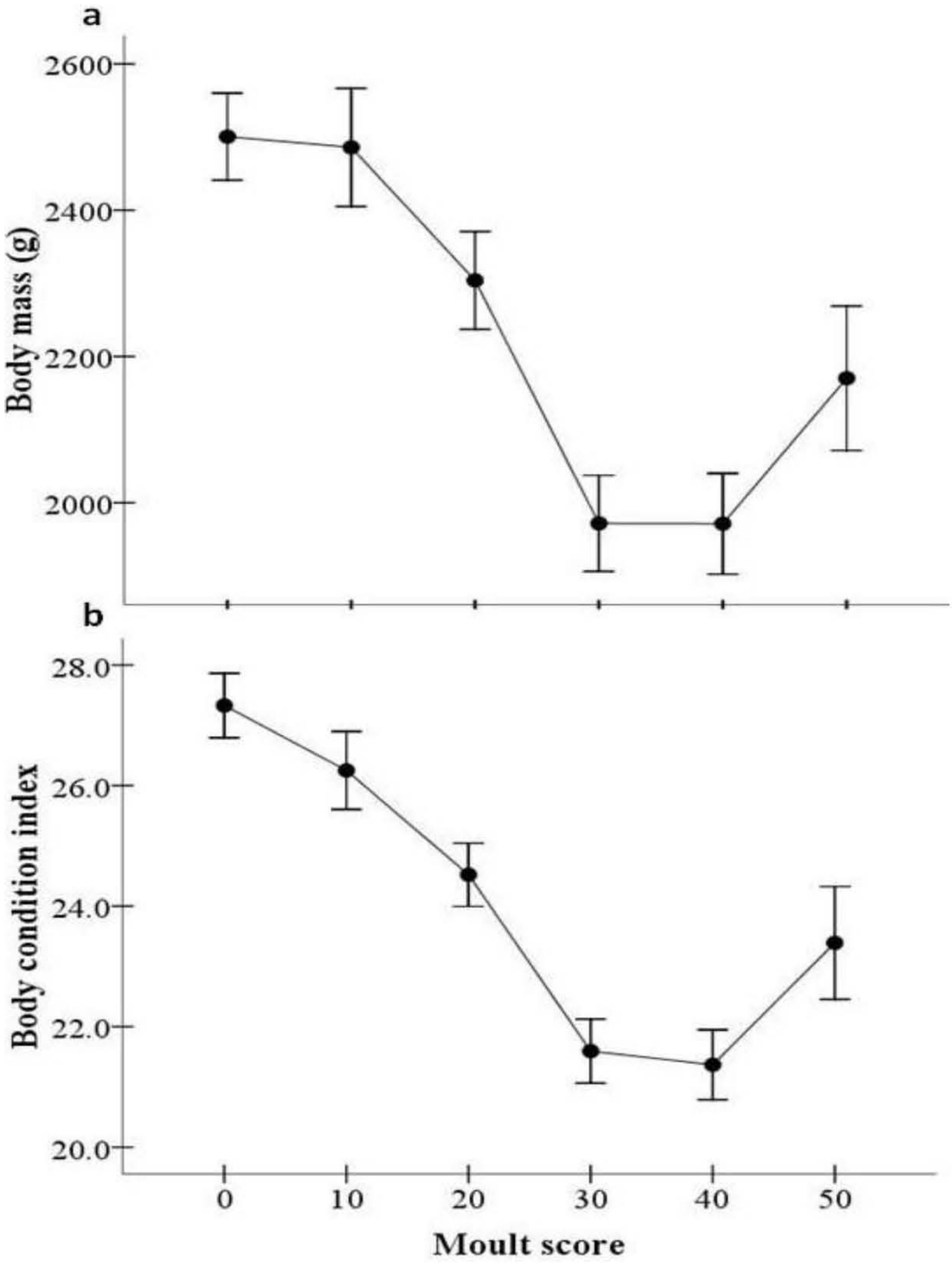

Figure 1. Mean mass (a) and body condition index (b) [± 95% C.I~~L~~] as a function of moult score for Egyptian Geese at Barberspan and Strandfontein combined.

Feather growth of captive birds

Of the 40 birds held in captivity, 22 were flightless and 18 were capable of flight. The latter had a P9 length of at least 158 mm (two-thirds grown). The mean P9 growth rate for all the moulting geese was 4.59 ± 0.32 mm.d^{-1}. P9 growth rate decreased (r = - 0.56; P < 0.0001) as feathers elongated (Fig. 2). The mean feather growth rate of flightless birds was 5.54 ± 0.32 mm.d^{-1} and for birds capable of flight the equivalent growth rate was 3.52 ± 0.46 mm.d^{-1}. Moulting geese are therefore likely to be flightless for at least 28.5 days. The mean P9 length of 221 non-moulting Egyptian Geese was 247.22 ± 1.31 mm. Therefore the average duration of moult based on the mean P9 growth rate for all moulting geese was 53.83 days, almost double the period of flightless moult.

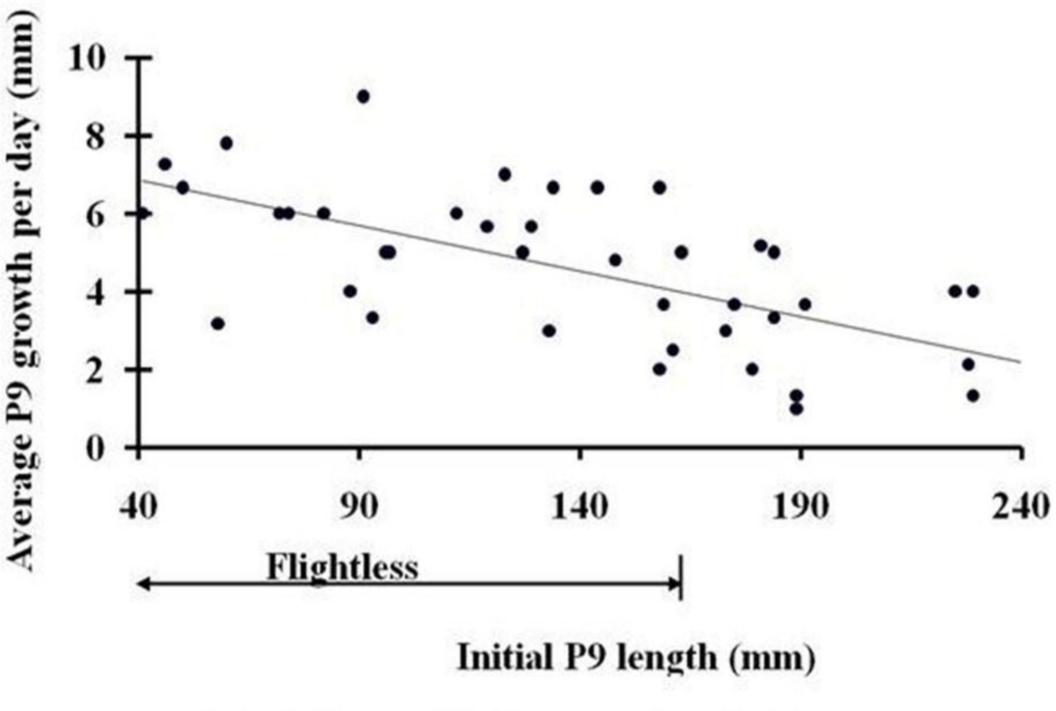

Figure 2. Average P9 increment per day (mm) as a function of initial P9 length for Egyptian Geese in captivity. Y = -0.023x + 7.778; r = - 0.65; F = 28.75; P < 0.0001.

Muscles and organs

Fat reserves with a score of 1 were recorded in three non-moulting geese and in one goose with new feathers. All other birds at all moult stages had no fat layer (score 0) and fat scores were not considered in subsequent analyses. The multivariate analysis suggests that masses of all organs measured, except the heart, varied significantly during moult (Table 1).

Table 1. MANOVA of organ weights during moult.

Organ Mass (g)	n	F	P
Heart	6	0.266	0.923
Gizzard	6	127.957	< 0.0001
Liver	6	58.932	< 0.0001
Pectoral muscles	6	10.616	< 0.0001

Pectoral muscle mass varied significantly during the moulting period. Non-moulting Egyptian Geese with very old flight feathers (i.e. about to moult) had high pectoral muscle mass, accounting for at least 26% of total body mass. Once moult started, pectoral muscle mass decreased and continued to do so until the flight feathers were at least one-third grown, after which pectoral muscle mass started to increase (Fig. 3a). In other words, the regeneration of pectoral muscles during moult began before birds started to gain overall body mass.

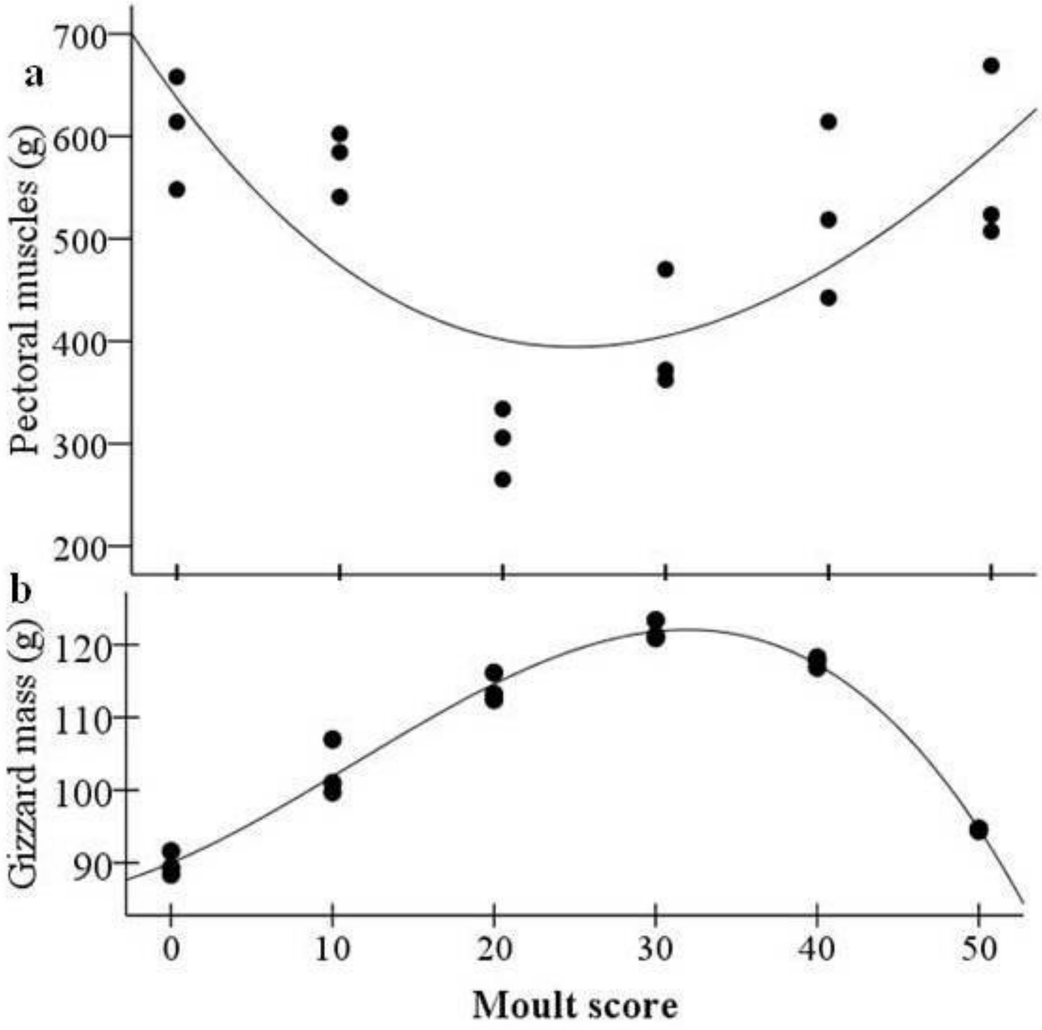

Figure 3. Changes in (a) wet pectoral muscle mass and (b) wet gizzard mass of Egyptian Geese at Strandfontein as a function of moult score. Fitted polynomial curves:

(a) $y = 0.3488x^2 - 18.165x + 630.63$; $r = 0.729$; $P = 0.003$

(b) $y = -0.001x^3 + 0.035x^2 + 0.94x + 89.98$; $r = 0.99$; $P < 0.0001$

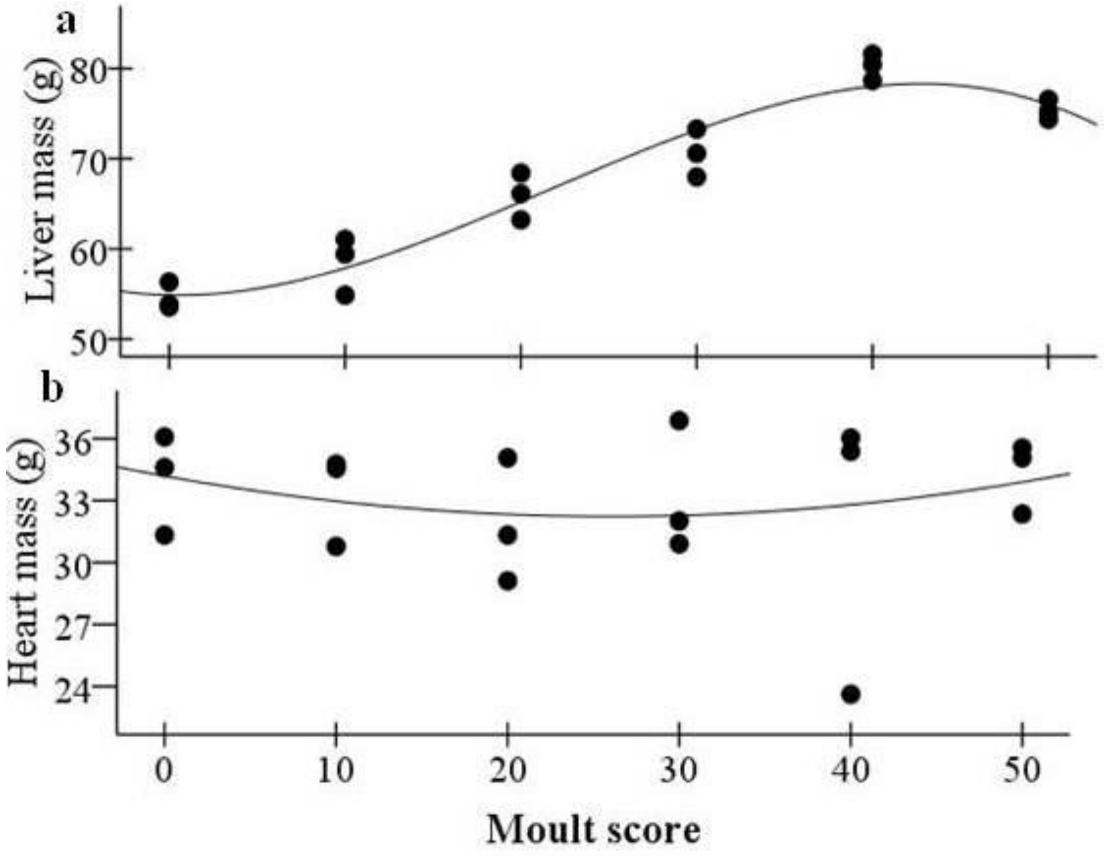

Figure 4. Changes in (a) wet liver mass and (b) wet heart mass of Egyptian Geese at Strandfontein as a function of moult score. Fitted polynomial curves:

(a) $y = -0.0063x^2 + 0.8102x + 53.013$ and $r = 0.9412$; $P < 0.0001$.

(b) $y = 0.003x^2 - 0.15x + 34.188$ and $r = 0.229$; $P = 0.667$

Gizzard mass started to increase soon after the onset of moult, peaking when the flight feather were two-thirds grown, after which gizzard mass again decreased. Liver mass increased significantly (P < 0.0001) as moult progressed, while heart mass remained unchanged (Fig. 4).

Discussion

Egyptian Geese exhibit substantial phenotypic flexibility during moult. Changes in both muscle and organ mass can be interpreted as strategies to balance energy and nutrient demands with the limitations imposed by reduced food availability during the flightless moult period.

The overall loss of body condition and mass (Fig. 1) that occurs from the onset of moult until birds are once again able to fly, albeit not well, is interpreted as a response to a combination of lost foraging opportunity (reduced foraging time and low-quality food) and the cost of feather growth (Murphy 1996). At face value, these findings support the conclusions of Hanson (1962) and Folk *et al*. (1966) that moulting ducks are nutrient stressed and lose body mass and condition as a result. However, a logical extension of this interpretation is that geese periodically starve to death during moult: there is, however, no evidence that this happens (Halse 1984; Milstein 1993). An equally sound interpretation of this pattern is that geese reduce body condition and mass to a safe minimum level, which they subsequently maintain. Once flight feathers are more than two-thirds grown and the birds are able to fly, they can venture farther from water to higher quality foraging areas, allowing them to increase body mass and improve overall body condition towards the end of the moult period. Similar patterns of changing body mass during moult have been documented for north-temperate waterfowl (e.g. Ankney 1984; Jehl 1997). The most parsimonious explanation for changes in body mass and condition appears to be that they represent strategic responses which simultaneously reduce metabolic demand (through reduction in flight muscle mass – Fig. 3a) and wing loading (by reducing mass). The combined effect of this is to reduce the period of flightless moult (heavier birds would have to wait for longer before the feathers had grown sufficiently to enable them to fly). The advantage of shortening the flightless moult period is that it speeds up birds' access to higher quality foraging areas which in turn enables them to be in better condition at the end of moult than would be the case had they been flightless for longer. Predation pressure could be an alternative explanation for adopting a strategy to reduce body mass and shorten the flightless period (Owen and Ogilvie 1979; Sjöberg 1986). I was unable to test this hypothesis explicitly in this study. However, Egyptian Geese probably have few natural predators in their southern African range: they are aggressive and are able to drive away large carnivores (Errington 1967; Howcroft 1968; Milstein 1993). In addition, they are only infrequently hunted for sport. This lack of extrinsic sources of mortality may contribute to explaining the species' major range expansion in southern Africa in the last 50-100 years (Okes *et al*. 2008).

The overall average daily primary feather growth rate of 4.59 ± 0.315 $mm.day^{-1}$ for Egyptian Geese in this study is less than that reported for most northern hemisphere ducks and geese (there are no southern African studies with which to compare these findings), although similar primary growth rates of 4.04 $mm.d^{-1}$ have been reported for Barrow's Goldeneye *Bucephala islandica* (van de Wetering and Cooke 2000). Much larger Mute Swans *Cygnus olor* have an average primary growth rate of 6.5 $mm.d^{-1}$ and Barnacle Geese *Branta leucopsis*, similar in size to Egyptian Geese, have a primary growth rate of 7.5 $mm.d^{-1}$ (Owen and Ogilvie 1979). Egyptian Geese therefore seem to take longer to complete their moult as compared to northern hemisphere waterfowl of equivalent size. However, the duration of the flightless period (*ca* 28-29 days) is shorter than most northern hemisphere ducks (32-36 days: e.g Owen and Ogilvie 1979; Pehrsson 1987; van de Wetering and Cooke 2000). Despite their long overall moult duration, Egyptian Geese are thus able to minimize the flightless period by reducing wing loading. They are able to fly with primaries that are only 67% grown as compared to northern hemisphere ducks (which maintain mass during moult) that can only fly once their primaries are at least 80% grown (Pehrsson 1987).

When flight feathers of Egyptian Geese were half grown, and before flight was possible, the pectoral muscles gradually started to increase in size, allowing sustained flight once the flight feathers were over two-thirds grown. Similar atrophy and hypertrophy of pectoral muscles has been recorded for moulting Greylag Geese (Fox and Kahlert 2005) and Black-necked Grebes (Gaunt *et al*. 1990) in the northern hemisphere. These findings also concur with Piersma and Lindström (1997) who predict that peak flight performance is sustained by enlarged pectoral muscles whilst temporary flightlessness will cause the pectoral muscles to shrink. Ankney (1979) further suggests that the proteins and nutrients released as the pectoral muscles atrophy may be used to supplement the growth of new flight feathers and the enlarged digestive organs.

The significant increase in the mass of the gizzard (which happens before the increase in pectoral muscle mass – Figs. 3a, b) is interpreted as an adaptation to low diet quality during moult (i.e. below the point at which birds can maintain good body condition). Similar changes in gizzard size in response to diet have been demonstrated in domesticated Japanese Quail *Coturnix japonica* (Starck 1999; Starck and Rahmaan 2003) and in Red Knots *Calidris canutus* (Dekinga *et al*. 2001). In both cases, gizzard mass increased within a week in response to hard-textured, low-protein forage and these changes reversed when birds were fed soft, high-quality food. The growth of the gizzard is a response to an increase in the work load involved in processing low-quality food (Starck 1999), as Egyptian Geese are forced to do early in the moult period. In the case of Egyptian Geese, gizzard mass started to decrease once birds were able to fly to higher quality foraging areas. This phenomenon has yet to be investigated for ducks in the northern hemisphere.

These findings cannot fully discount the alternative hypothesis that the observed patterns are driven purely by the high energetic costs of feather growth. If the patterns were driven exclusively by stress (i.e. exogenously), it is possible to explain the pattern of increasing then decreasing gizzard mass as nothing more than a physiological response to forage quality (and one which has been demonstrated experimentally; see Starck 1999; Dekinga *et al*. 2001; Starck an Rahmaan 2003). It is also possible to explain why overall body mass decreases up to the time that birds are able to exploit high-quality food, because a combination of a poor nutritional environment coupled with the energetic demands of feather growth results in a negative energy balance (Murphy 1996). What cannot be explained by the 'feather growth cost' hypothesis is the allocation of increasingly scarce resources to pectoral muscles (i.e. increasing flight muscle mass while overall body mass continues to fall) before birds have access to high-quality food. This strongly implies an anticipation of need (i.e., a strategy) rather than an inevitable response to stress. Indeed, based on low-quality foraging conditions until the time the birds are able to fly, it is very difficult to understand how this pattern could be explained by the feather growth cost hypothesis. The findings of the study are consistent with the hypothesis that moulting Egyptian Geese adjust their mass and body condition to minimize the flightless period; i.e. the phenotypic changes observed represent an adaptive strategy, *sensu stricto*.

The problem of reducing the flightless period is fundamental to all ducks that moult on water but graze on land. Grazing ducks in the northern hemisphere have an accelerated feather growth rate that they maintain by using stored fat reserves (e.g. Ankney and MacInnes 1978; Fox and Kahlert 2005). Unlike northern hemisphere ducks, Egyptian Geese do not deposit fat reserves prior to moult and they have a slower feather growth rate. However, they trade off body mass, condition and muscle size for a reduction in wing loading that in turn shortens the flightless period.

In summary, unlike north-temperate ducks, moulting Egyptian Geese in South Africa undergo substantial phenotypic changes during moult, including asynchronous changes (e.g. flight muscle mass increasing while body mass decreases). This phenotypic plasticity appears to have evolved to allow moult to be completed as rapidly as possible in environments characterized by stochasticity rather than the predictability which prevails at boreal latitudes. Specifically, the moult strategy of Egyptian Geese is geared towards minimizing the duration of the flightless period, a strategy that could not have been predicted based on prevailing northern hemisphere paradigms. These findings offer new insights into duck life-history strategies in different environments, especially stochastic environments.

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CHAPTER 3

Body mass and pectoral muscle size fluctuations in African ducks during moult

Abstract

Body mass and organ mass dynamics during flight-feather moult vary among waterfowl species. To test for the existence or extent of interspecific variation in Afrotropical waterfowl body-mass fluctuations during flight-feather moult, I investigate how body mass and pectoral muscle size of Spur-winged Geese and South African Shelducks change during flight-feather moult and how body mass varies in Yellow-billed Ducks, Red-billed Teals and Southern Pochards. My results demonstrated that; (a) Spur-winged Geese and South African Shelducks underwent atrophy and subsequent regeneration of the pectoral muscles, while their body mass decreased at the onset of moult and then stabilised from the time when flight feathers were two-thirds grown until moult was completed; (b) body mass of Yellow-billed Ducks and Red-billed Teals decreased from the onset of moult until the mid-point but thereafter increased rapidly, returning to pre-moult levels by the time moult was completed; and (c) Southern Pochard (a only diving duck in the study) gradually lost mass from the start of moult almost until moult completion, at which time mass increased slightly. These findings imply that during flight-feather moult, Afrotropical waterfowl (except for Pochards) exhibit different fluctuations in body mass and pectoral muscle size. The need for such mass fluctuations is likely precipitated by a combination of a) environmental aridity and stochasticity; b) predation risk; and c) the need to relocate to high-quality feeding areas as soon as possible.

Introduction

Waterfowl (Anatidae) moult all their flight-feathers simultaneously, rendering them flightless for a period of 3-4 weeks (Hohman *et al*. 1992). During this flightless period waterfowl need water of sufficient depth for foraging and predator avoidance (Geldenhuys 1981) and many waterfowl undertake a pre-moult migration to permanent wetlands that may be hundreds of kilometres from their breeding sites (Salomonsen 1968). Being flightless confines waterfowl to areas where food depletion is possible unless they can swim or walk to alternative sources of food. Hence, the potential exists for moult to become energy limited (Hanson 1962; Lindström *et al*. 1993; Baldassarre and Bolen 2006; Piersma and Lindström 1997), especially in the case of terrestrial grazers, who cannot risk wandering too far from water (Hanson 1962; Folk *et al*. 1966; Murphy 1996). For this reason, flight-feather moult is a potential bottleneck in the life histories of waterfowl. As a result many waterfowl adjust body mass and internal organ mass as a strategy to reduce both energy costs and the duration of the flightless period (Brown and Saunders 1998; Fox and Kahlert 2005; Fox *et al*. 2008; Ndlovu *et al*. 2010; Chapter 2).

Body mass and organ mass dynamics during flight-feather moult vary among waterfowl species (Douthwaite 1976; Ankney 1979 Brown and Saunders 1998; Fox *et al*. 1998), and in some cases within the same species at different locations (e.g., Greylag Goose *Anser anser* - Fox *et al*. 1998; Fox and Kahlert 2005). Some species lose body mass during moult, which has been interpreted as an adaptation to reduce wing-loading and minimise the duration of the flightless period, allowing birds to fly well before the remiges are fully grown (Douthwaite 1976, Pehrsson 1987, Sjöberg 1988, Brown and Saunders 1998). Other studies, however, have reported no significant body-mass change in ducks during flight-feather moult (e.g. Ring-necked Duck *Aythya collaris* - Hohman *et al*. 1988; and Common Scoter *Melanitta nigra* - Fox *et al*. 2008). Few studies have addressed flight-feather moult in Afrotropical waterfowl and most of these have considered only body-mass dynamics during moult (e.g. Red-billed Teal *Anas erythrorhyncha* - Douthwaite 1976; South African Shelduck *Tadorna Cana* - Shewell 1959, Geldenhuys 1983; and Spur-winged Goose *Plectropterus gambensis* - Halse and Skead 1983). Dean and Skead (1979) studied body mass changes in South African Shelduck, Yellow-billed Duck *Anas undulata* and Red-billed Teal during flightless moult and reported a gradual, linear decrease in body mass. However visual inspection of their data suggests a slight increase in body mass towards the end of flight-feather moult. The changes that occur in body mass, pectoral muscle size and the masses of internal organs (gizzard, liver and heart) of southern African ducks during flight-feather moult had, until recently, never been explored. A detailed study of moulting Egyptian Geese *Alopochen aegyptiaca* (Chapter 2) revealed that they exhibit substantial phenotypic flexibility during flight-feather moult, adjusting not only their body mass and condition, but also their energy allocation to pectoral muscles and internal organs in such a way as to lower wing-loading and minimise the flightless period. Their strategy appears to be similar to patterns documented in the northern hemisphere for moulting Greylag Geese (Fox and Kahlert 2005) and (captive) Barnacle Geese *Branta leucopsis* (Portugal *et al*. 2009).

The phenotypic flexibility of Egyptian Geese raises questions about the body mass and pectoral muscle dynamics of other, poorly studied southern African ducks during flight-feather moult. In particular, it is unclear whether Egyptian Geese pursue a ‘typical’ moult strategy or an unusual one. Some interspecific variation in waterfowl body-mass changes during moult is expected, but the extent to which body-mass change is similar or contrasting among waterfowl occupying the same environment, particularly in tropical and subtropical areas that are not strongly seasonal, is still unknown (Petrie and Rogers 2004). Interspecific differences in body-mass changes during flight-feather moult can be attributed to mass-related differences in metabolic rate, which predicts that small birds with the highest mass-specific metabolic rates will have the highest moult costs and hence lose body mass most rapidly during flight-feather moult (Lindström *et al*. 1993; Hoye and Buttermer 2011). With wing-loading taken into account, however, mass-related differences may cause the opposite response. For a given wing shape, wing-loading increases as a function of body mass$^{0.33}$ (Greenewalt 1962). Consequently, smaller birds have inherently lower wing-loading than larger ones, which means that larger birds have to lose proportionately more mass in order to be able to fly before their remiges are fully grown (Marden 1987).

Inter- and intraspecific differences in body mass during flight-feather moult have been related to variation in species' abilities to satisfy the energy demands of feather synthesis as a consequence of differences in foraging mode (e.g. Fox *et al*. 1998). Because body-mass change during flight-feather moult is strongly driven by the balance between energy demand and supply (Lindström *et al*. 1993), it might be expected that ducks that have a wholly aquatic lifestyle (diving and dabbling species), and thus do not have to come ashore to forage during flightless moult and face concomitant risks of predation, show the least adjustment in body condition during flight-feather moult. For instance, dabbling female Mottled Ducks *Anas fulvigula* (Moorman *et al*. 1993) and diving Ring-necked Ducks (Hohman *et al*. 1988) in the United States exhibit no body-mass change or pectoral muscle regeneration during flight-feather moult. By contrast, dabbling Red-Billed Teals *Anas erythrorhyncha* (Douthwaite 1976), Common Teals *A. crecca* (Sjöberg 1988), and Mallards *A. platyrhynchos* (Panek and Majewski 1990) all lose mass during moult. Terrestrially foraging ducks (e.g. Egyptian Geese) and ducks that partly graze on land and partly dabble in water (e.g. South African Shelduck) might be predicted to exhibit greater atrophy of body mass and pectoral muscle size during flight-feather moult than would exclusively dabbling and diving species, especially given that most southern African moulting locations are not adjacent to high-quality grazing pasture (Young and Boag 1982; Chapter 2). The hypothesis that foraging mode may play a role in determining the level of body mass and pectoral muscle change displayed by ducks during flight-feather moult has not previously been explored.

To test for the existence or extent of interspecific variation in body-mass fluctuations during flight-feather moult, I investigated how body masses of South African Shelducks, Spur-winged Geese, Yellow-billed Ducks, Southern Pochards, and Red-billed Teals change during flight-feather moult. Whether pectoral muscle size changes at this time was also investigated in South African Shelducks and Spur-winged Geese. These five waterfowl species are all ducks (Anatinae) despite their common names. The large Spur-winged Goose (mean mass 4500 g) and medium-sized South African Shelduck (mean mass 1260 g) are mixed terrestrial/aquatic foragers (Hockey *et al*. 2005). The smaller Yellow-billed Ducks (960 g) and Red-billed Teals (645 g) are mainly aquatic dabbling ducks but will readily eat corn and grass (Hockey *et al*. 2005). Southern Pochards (840 g) are exclusively aquatic-foraging, diving ducks (Hockey *et al*. 2005).

The study tests two overarching hypotheses:

1. If the level of body mass and pectoral muscle size variation during flight-feather moult in these species is driven by environmental conditions and energetic needs (i.e. food availability balanced against predation risk), then ducks moulting in stochastic environments adaptively adjust their body mass and pectoral muscle size to reduce wing loading and minimise the flightless period, as was found with Egyptian Geese (Chapter 2). I therefore predicted that (a) all five ducks will exhibit a similar pattern of body-mass and pectoral muscle size change during flight-feather moult; and (b) this pattern will be analogous to that documented for Egyptian Geese.
2. Flight-feather moult is not costly to southern African ducks, and hence there are no significant changes in body condition or pectoral muscle size during moult.

The study also partly addresses another competing prediction that has been proposed to link changes in body mass and pectoral muscle size (phenotypic flexibility) during moult to foraging mode and energetics:

- The extent of body mass and pectoral muscle size adjustments is driven solely by energetic constraints, given that foraging mode (terrestrial grazing *vs* aquatic dabbling *vs* diving) influences both the availability and apportionment of energy and ultimately the changes in body mass and during the moulting period.

Materials and methods

Data were collected at two sites in South Africa (see Chapter 2). Forty adult Spur-winged Geese were sampled from Strandfontein Wastewater Treatment Works during March 2009. Forty-eight adult South African Shelducks were caught in December 2007 and December 2008 at Barberspan Nature Reserve. Two hundred and fifty-two Yellow-billed Ducks (197 at Barberspan and 55 at Strandfontein) and 439 Red-billed Teal (380 at Barberspan and 59 at Strandfontein) were caught between March 2007 and February 2009. Unpublished moult and mass data for a further 297 Yellow-billed Ducks, 652 Red-billed Teals and 93 Southern Pochards were obtained from the Barberspan Research Station ringing records of 1977, giving total sample sizes of 549, 1091 and 93 for these three species, respectively (Barberspan Research Station, 1977).

Ducks were caught using maize-baited, walk-in traps placed near the water's edge. Mass was measured to the nearest gram using a spring balance. Moult, body condition (see below), total head and tarsus length (from the end of the calcaneal ridge to the base of the last complete scale before the toes diverge) were recorded for all ducks. Head and tarsus lengths were measured with Vernier callipers to the nearest 0.1 mm.

Moult scores were recorded following de Beer *et al*. (2000). For each of the ten primary feathers, moult was scored using a six-point scale: 0 = fully grown old (worn) feather, 1 = feather missing or in pin, 2 = feather emerging from sheath up to 33% grown, 3 = new feather 33-67% grown, 4 = new feather from 67% to fully grown with remains of the sheath still visible, 5 = new, fully grown feather with no remaining traces of a sheath. Scores for each feather were summed, giving a total moult score. Because ducks undergo synchronous moult of the remiges, six distinct moult stages (i.e. 0, 10, 20, 30, 40 and 50, with no in-between scores) were recognizable.

A measure of body condition for Spur-winged Geese and South African Shelduck was derived from the Body Condition Index (BCI) developed by Ndlovu *et al*. (2010) which corrects body mass for structural size differences. It was not possible to calculate BCIs for Yellow-billed Ducks, Red-billed Teals and Southern Pochards from the Barberspan ringing records from the 1970s, because these contained only mass and moult score: instead raw mass data were analysed for these three species.

Given that sample sizes for Spur-winged Geese and South African Shelducks were small (40 and 48, respectively), a non-destructive Breast Score (BS) was used as a surrogate measure for pectoral muscle size. Gregory and Robins (1998) used a similar method for determining the body condition of domestic chickens. The palm and fingers of the right hand were used to palpate and grade the protuberance of the keel and the convexity or concavity of the pectoral muscles. The size of the pectoral muscle as felt by hand was scored using a five point scale: 1 = lean - prominent ridge on the keel with slightly concave pectoral muscle, ribs can be felt; 2 = slightly lean, with flat pectoral muscles, ridge can be felt; 3 = average, with a moderately convex pectoral muscle; 4 = plump - convex pectoral muscle, but ridge discernible; and 5 = very plump - ridge cannot be felt. All measures of pectoral muscle size were made by me and were highly repeatable. Barberspan ringing records did not have pectoral muscle scores nor tarsus, head or culmen lengths. Only body mass and moult scores were considered for Yellow-billed Duck, Red-billed Teal and Southern Pochard.

Statistical analysis

Simple linear interpolations drawn between mean values were used to describe changes in body condition and pectoral muscle score as a function of moult score. One-way analysis of variance (ANOVA) was used to test for the effects of moult stage on body condition and pectoral muscle scores. Hypotheses were tested at the 5% level of significance and all analyses were carried out using the package SPSS 17 (SPSS Inc., Chicago IL 2008).

Results

Although present at both study sites, Spur-winged Geese were caught only at Strandfontein in March 2009. Twenty-three of the 40 birds caught were in moult. Body Condition Index varied significantly (F = 3.18, P = 0.018, n = 40) over the moult period, and started to decrease as soon as the flight feathers were shed, continuing to do so until the new flight feathers were about half grown. Thereafter BCI stabilised and then increased, but not significantly, until the end of moult (Fig. 1a). Breast Score also varied significantly during moult (F = 4.09, P = 0.005, n = 40), decreasing from the onset of moult until new feathers were two-thirds grown, after which BS increased up to the point at which feathers were fully grown (Fig. 1a).

Eighteen of the 48 South African Shelducks that were caught were in moult, with moult scores of between 10 and 40. The BCI of these birds varied significantly during the moult period (F = 10.88, P < 0.0001, n = 48). Pre-moulting birds had a higher BCI than post-moulting birds, and BCI started to decrease as soon as the remiges were shed. The decrease in BCI continued until the new flight-feathers were half grown, after which BCI stabilised until the end of moult. The slight increase in body mass of birds with moult scores >40 was statistically insignificant (Fig. 1b). Mean BS also varied significantly during moult ($F = 3.99$, $P < 0.005$, $n = 48$). Body Score increased before the flight feathers were dropped and thereafter decreased until new flight feathers were between one-third and two-thirds grown, after which BS increased significantly (Fig. 1b).

The mean body masses of Yellow-billed Duck and Red-billed Teal varied significantly ($F = 58.58$, $P < 0.0001$, $n = 549$; $F = 94.19$, $P < 0.0001$, $n = 1091$, respectively) over the moult period. Mean body mass of both species started to decrease at the onset of moult and continued to do so until the flight feathers were two-thirds grown (moult score 30), after which mass started to increase until it reached the level of non-moulting birds (Figs. 2a and b).

The mean body mass of Southern Pochards also varied significantly ($F = 26.7$, $P < 0.0001$, $n = 93$) during moult. Mean body mass started to decrease at the onset of moult and continued to do so until feathers were more than two-thirds grown (moult score 40), after which mass started to increase (Fig. 2c). Body mass of pre-moulting birds was significantly less than that of post-moulting birds ($P < 0.005$, Fig. 2c).

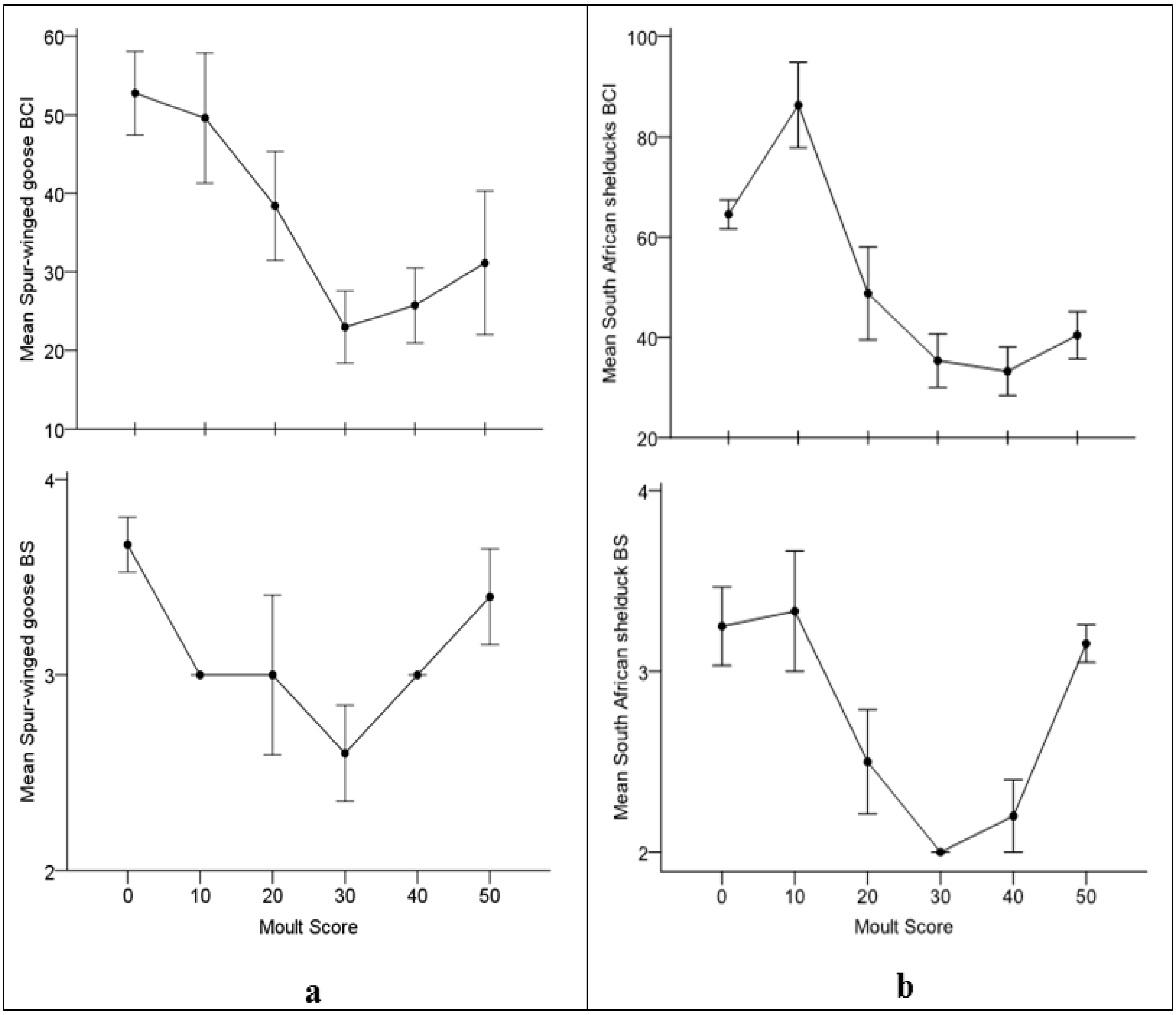

Figure 1. Mean Body Condition Index (± 95% C.L) and Breast Score as a function of moult score for Spur-winged Geese at Strandfontein (a) and South African Shelducks at Barberspan (b).

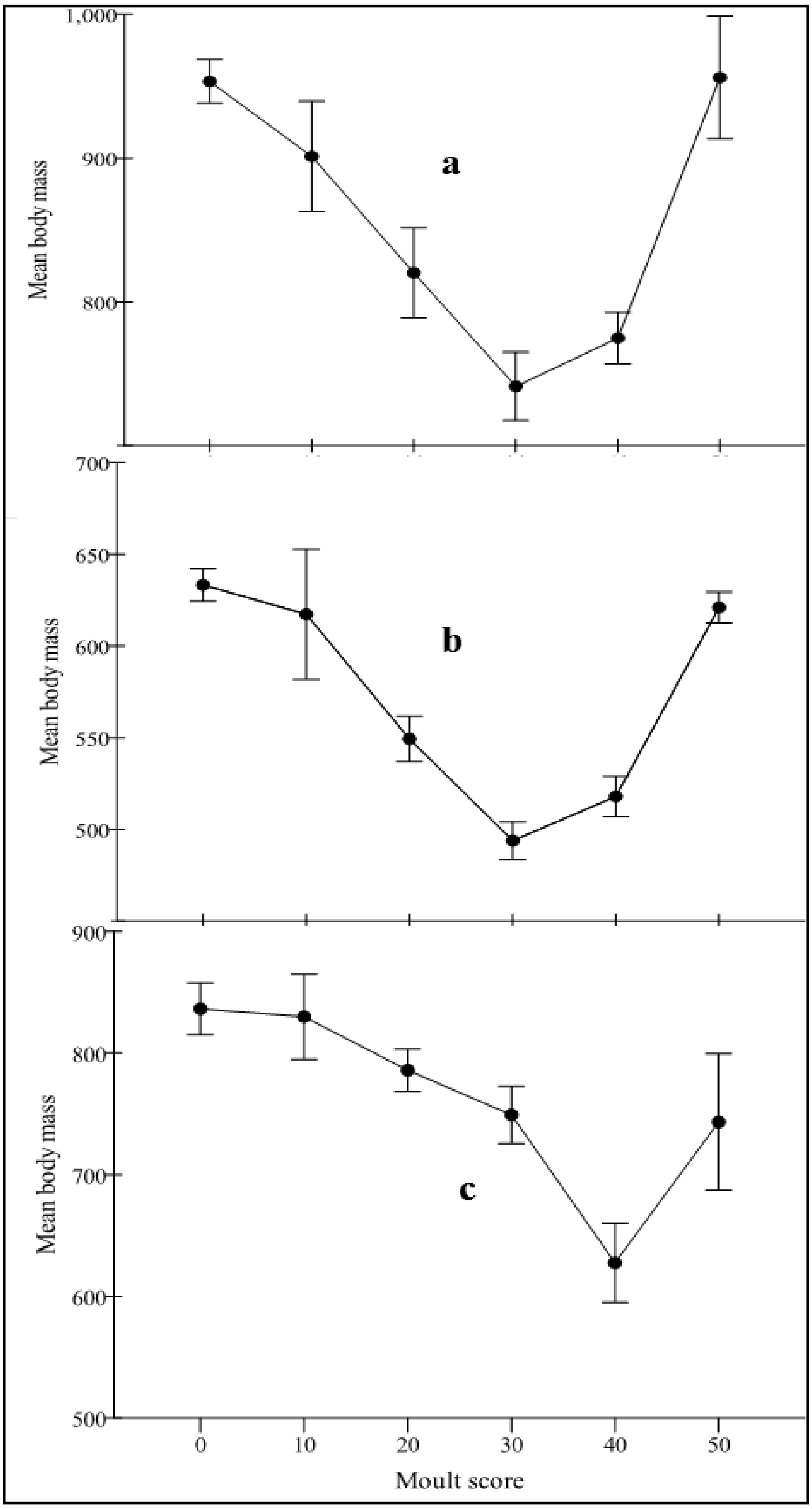

Figure 2. Mean body mass (± 95% C.L) as a function of moult score for Yellow-billed Ducks at Barberspan and Strandfontein combined (a), Red-Billed Teals at Barberspan and Strandfontein combined (b) and Southern Pochards at Barberspan (c).

Discussion

Spur-winged Geese and South African Shelducks underwent atrophy and subsequent regeneration of the pectoral muscles, while their body masses decreased at the onset of moult and later stabilised from the time when flight feathers were two thirds grown until moult completion. Body masses of Yellow-billed Ducks and Red-billed Teals decreased from the onset of moult until the mid-point but thereafter increased rapidly, returning to pre-moult levels by the time moult was completed. Southern Pochards gradually lost mass from the start of moult almost until moult completion, at which time mass increased slightly.

Spur-winged Geese, South African Shelducks, Yellow-billed Ducks and Red-billed Teals did not appear to fast during flight-feather moult because birds were observed foraging during counts and all moulting ducks in this study were captured using food-baited traps placed at the water's edge. These southern African ducks thus forage actively during flightless moult.

Outside the moulting season both Spur-winged Geese and South African Shelducks forage mostly in crop fields, grasslands and eutrophic shallow wetlands (Geldenhuys 1977). The change of environment to otherwise low-nutrient, moulting pans presents fewer foraging opportunities. Of these two species, the smaller South African Shelduck has higher mass-specific metabolic demands than the much larger Spur-winged Goose (MacNab 2003). South African Shelducks thus require more energy as a proportion of body mass while moulting and appear to deposit reserves in anticipation of an energetically costly period (Fig. 1b), the only one of the five species to do so. Most southern Africa waterbirds do not deposit reserves in anticipation of an energetically costly period. This is purely a response to environmental predictability/unpredictability. Ducks in the northern hemisphere face the ever-present risk of freezing conditions, making their food unavailable. This is not a risk faced by the birds in the south, ergo there is no need to carry (costly) metabolic insurance.

Apart from this difference (shelducks depositing reserves prior to moult) between Spur-winged Geese and South African Shelducks, patterns of changing body condition and pectoral muscle size of these two species during the moult period were essentially similar, mirroring patterns of phenotypic change exhibited by Egyptian Geese at the same sites (Chapter 2). Both species lost body condition and experienced reduced pectoral muscle size from the onset of moult until feathers were two-thirds grown. After the mid-point of moult (moult score 30), body condition of both species stabilised (with subsequent small, non-significant increases). Pectoral muscles were also smallest at the mid-point of moult, after which they grew rapidly (and significantly), returning to pre-moult level by the time flight-feather moult was completed (Figs. 1a and b). The initial atrophy and subsequent regeneration of the pectoral muscles in both species mirrors the patterns shown by Greylag Geese (Fox and Kahlert 2005) moulting in the northern hemisphere. It is interesting to note that even though pectoral muscles account for about 25% of total body mass (Chapter 2), the regeneration of pectoral muscles that occurred in Spur-winged Geese and South African Shelducks after the mid-point of moult did not translate into a significant increase in overall body mass (Figs. 1a and b). Pectoral muscle mass was scored on a five-point (BS) scale: a single increment on this scale thus represents approximately a 5% increase in overall body mass (assuming the mass of other organs remains constant). Thus, a significant increase in BS (Figs. 1a, b) is not necessarily reflected in a significant increase in BCI (Figs 1a, b). Alternatively, it is possible that both Spur-winged Geese and South African Shelducks experienced gradual atrophy of other body organs (e.g. Piersma and Lindström 1997) or locomotory muscles (e.g. Ankney 1984) across the moult period, as did Egyptian Geese (Chapter 2), counteracting the gain in weight of the pectoral muscles.

Decreases in body mass and pectoral muscle size during the first half of the moult period represent strategic responses which simultaneously reduce a) the metabolic costs of sustaining flight muscles during flightless moult (Piersma and Lindström 1997), and b) wing-loading (Sjöberg 1988; Brown and Saunders 1998). The net effect is to shorten the period of flightless moult (heavier birds would have to wait for longer before the feathers had grown sufficiently to enable them to fly). The advantages of shortening the flightless moult period are: a) reducing exposure time to predators, and b) minimising time spent at low-quality foraging areas.

Yellow-billed Ducks, Red-billed Teals and Southern Pochards did not appear to store fat reserves prior to moult (Figs 2a, b, c). The foraging mode hypothesis I proposed predicts that dabbling and diving ducks have less need to store such reserves than do terrestrial grazers because the medium (water) in which they both moult and forage is the same. In the northern hemisphere, diving Common Scoters (Fox *et al*. 2008) and dabbling male Mallards (Young and Boag 1982) maintain a constant body mass during moult, but the same is not true of the diving and dabbling ducks in South Africa. Both Yellow-billed Ducks and Red-billed Teals (dabbling ducks) lost mass during the first half of moult and Southern Pochards (divers) continued to lose mass until their flight-feathers were almost fully grown. Similar patterns of mass loss have been documented for dabbling Common Teal *Anas crecca* in Sweden (Sjöberg 1988).

For Yellow-billed Ducks and Red-billed Teals, body masses were lowest around the mid-point of moult, but thereafter increased rapidly, with the birds regaining pre-moult weights by the time moult was completed (Figs 2a and b). Egyptian Geese moulting at the same sites follow the same pattern, interpreted as a strategy to reduce wing-loading and enable birds to fly with partly grown flight feathers (Sjöberg 1988; Brown and Saunders 1998; Chapter 2). Southern Pochards, by contrast, gradually lost mass through almost the entire moult period, with pre-moulting birds being significantly heavier than post-moulting birds. Without knowledge of how mass loss is apportioned across the body (e.g. whether it reflects solely a change in the size of the pectoral muscles) it is difficult to distinguish whether this gradual decrease in mass is a 'strategy' *per se* or a passive response to energetic stress. Fox and King (2011) speculated that body mass loss in Northern Pochards *Aythya ferina* and Tufted Ducks *A. fuligula* during moult was a result of metabolism of endogenous fat reserves as a strategy to complete flight-feather moult rapidly without relying entirely on energy derived from foraging. However, without clear evidence from destructive sampling, I could not test this hypothesis for Southern Pochards or for Red-billed Teals.

Taken together, the results of this study suggest that no single hypothesis can fully explain the interspecific differences in the moult strategies of southern African waterfowl as reflected in changes in body and pectoral muscle conditions. Chapter 2 highlighted the importance of environmental aridity and stochasticity in determining patterns of body mass, pectoral muscle size and the masses of internal organs during moult, concluding that body mass and pectoral muscle size changes during moult by Afrotropical ducks are environmentally related. All African ducks considered in this study underwent a decrease in mass from the time when flight-feathers were dropped until flight-feathers were two-thirds grown, thereby reducing wing-loading and shortening the flightless period. Although this general pattern was true of all five species in this study, and of Egyptian Geese (Chapter 2), there were subtle interspecific differences that may be linked (in an as yet unknown way) to differences in foraging modes and possibly in the quality of the moulting habitat (Fox and Kahlert 2005). The levelling off in body mass of the Egyptian, Spur-winged Geese and South African Shelduck at the stage when flight feathers are at least two-thirds grown may reflect their ability to exploit novel food resources that they previously could not reach during true flightlessness

The most parsimonious explanation of observed body-mass fluctuations in Spur-winged Geese, South African Shelducks, Yellow-billed Ducks and Red-billed Teals appears to be that they cause a reduction in wing-loading and permit flight-feather moult to be completed as rapidly as possible. The need for such a strategy is likely precipitated by a combination of (a) environmental aridity and stochasticity; (b) predation risk; and (c) the need to relocate to high-quality feeding areas as soon as possible. Nevertheless, Southern Pochards displayed a unique, steady body-mass loss during moult and this cannot be described as an adaptation to reduce wing-loading or a strategy to ensure a rapid completion of moult. Rather, it is suggestive of energetic stress during moult. Thus, although this study shed light into the physiology of Afrotropical waterfowl during flight-feather moult, without knowledge of how other muscle groups like legs and breast fluctuated during moult we cannot fully understand the significance of body-mass fluctuations in Spur-winged Geese, South African Shelduck, Yellow-billed Ducks, Red-billed Teals and Southern Pochards during flight-feather moult.

Priority species for further study on body-mass and organ-mass dynamics during flight-feather moult (phenotypic flexibility) are Comb Duck *Sarkidiornis melanotos* (a nomadic species, favouring ephemeral pans – Hockey *et al*. 2005); Maccoa Duck *Oxyura maccoa* (a species with specialised habitat preference, and thought to have a moult strategy similar to that of the Southern Pochard, but with two flightless moults annually – Hockey *et al*. 2005); White-faced Duck *Dendrocygna viduata* (also a nomadic species, but which aggregates to moult in winter; and is also capable of flight before the remiges are fully grown – Petrie and Rogers 2004); and Hottentot Teal *Anas hottentota* (which are thought to be similar to the Red-billed Teal – Hockey *et al*. 2005).

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CHAPTER 4

Interactions of environmental conditions and the timing of breeding and moult in waterfowl

Abstract

Moult in waterfowl is expected to occur when energy is not needed for long-distance movements and reproduction, and when a safe moulting habitat is available. The few studies that test these expectations are biased towards northern hemisphere species and show close links between the end of breeding and the onset of moult. I studied the interactions of environmental conditions and the timing of breeding and moult in waterfowl. I tested the hypothesis that moult is the most energy-costly stage of the annual cycle and all other life history stages are built around the timing of moult. I investigated how the timing of rainfall relates to the timing of breeding and moult in Egyptian Goose *Alopochen aegyptiaca*, Spur-winged Goose *Plectropterus gambensis*, South African Shelduck *Tadorna cana*, Yellow-billed Duck *Anas undulata* and Red-billed Teal *A. erythrorhyncha* at two sites in South Africa with opposite rainfall regimes (one summer, one winter). I then incorporated published data to compare and contrast the relative timing of breeding and moult in southern hemisphere (southern African and Australian) waterfowl with northern hemisphere (European and North American) species. My results showed that southern African waterfowl breed in the wet season and moult during the dry season. South African Shelduck was an exception, breeding in the dry season and moulting during the wet season in the summer-rainfall area. There was also a long lag period between peak breeding and peak moult in southern hemisphere waterfowl species, the longest lag being that of birds in the summer-rainfall area. By comparison, northern hemisphere waterfowl species breed and moult during the warm season, with a shorter lag period between peak breeding and peak moult compared to southern hemisphere species. I concluded that waterfowl in southern Africa (with the exception of South African Shelduck), south-east Australia, Europe and North America time their breeding period to coincide with peaks in the availability of both food and breeding sites. Northern hemisphere species moult when temperatures are warm, and before food and aquatic habitats approach their winter minima. By contrast, southern-hemisphere waterfowl

delay the onset of moult until the dry season, opting to moult when both food and aquatic moulting habitats are few.

Introduction

Waterfowl have evolved to survive in diverse environments with different spatio-temporal variability in climatic conditions (Owen and Black 1990; Baldwin and Lovvorn 1994). A significant amount of research has been dedicated to understanding how environmental factors influence the timing of reproduction and migration in north-temperate waterfowl. Moult, however, as a key component of the annual cycle, has been less well studied, especially in southern hemisphere waterfowl (Owen and Black 1990). The risks of predation and starvation are significant for a duck during its flightless moult (Geldenhuys 1981a; Baldassarre and Bolen 2006). As a result, moulting ducks require habitats with sufficient food, and extent and depth of water to last for the duration of flight-feather moult. For this reason, flight-feather moult (termed ‘moult’ from here on) may be a potential bottleneck in the life histories of waterfowl and may be a key life-history stage around which other life-history events are scheduled.

Moult in waterfowl is expected to occur when energy is not needed for other life-history stages, particularly long-distance movements and reproduction, and when a safe moulting habitat and food are available simultaneously (Owen and Cook 1977; Holmgren and Hedenström 1995). The few studies that test these expectations are biased towards northern hemisphere species and show close links between the end of breeding (when young are independent) and the onset of moult (Joensen 1973; Mathiasson 1974; Dean 1978; Geldenhuys 1981a; Austin and Fredrickson 1986; Lovvorn and Barzen 1988; Vrtiska *et al*. 1997; Kear 2005). The availability of habitat and food in north-temperate and Arctic regions is determined primarily by large and highly predictable seasonal variations in temperature and day length. North-temperate waterfowl time their reproduction and moult stages to exploit this brief warm season (Wingfield *et al*. 1992; Svensson 1995; Piersma and Drent 2003; Bêty *et al*. 2004). In contrast, environmental conditions (both seasonal and annual) in the semi-arid regions of the southern hemisphere are regulated primarily by rainfall that is highly variable in space and time (Tyson and Preston-Whyte 2000), leading to greater environmental unpredictability than birds experience at high northern latitudes. Because the availability of habitat and food for waterfowl in semi-arid regions is determined ultimately by such rainfall events, there is substantial spatio-temporal variability in the availability of suitable habitat and food resources for both reproduction and moult (Siegfried 1974). Given this stochasticity, southern hemisphere waterfowl (especially those in semi-arid regions of South Africa and Australia) may use a variety of environmental cues to fine-tune their breeding and moult 'timetables' (Herremans 1999; Herrmann *et al*. 2004).

In the southern hemisphere, rainfall influences the availability of (1) food resources prior to moult (which are essential to ensure birds commence flight-feather moult in good condition); and (2) adequate water at wetlands that are suitable moult sites (Herrmann et al. 2004). The hypothesis that rainfall controls the timing of moult is not new (Geldenhuys 1981a), but to date has not been tested rigorously because of a lack of data on the seasonality of moult in most species, and especially its degree of synchrony.

In this study I test the hypothesis that moult is the most energetically costly stage of the annual cycle and that other major life-history events are built around this. Moult will therefore coincide with the time of year when food and water bodies are most abundant (Owen and Cook 1977; Holmgren and Hedenström 1995). An alternative hypothesis predicts that reproduction is more energetically costly than moult (Baldassarre and Bolen 2006) and hence the timing of breeding is the one that coincides with peak abundance of food and aquatic habitat. Moult is expected to follow soon after breeding (Joensen 1973; Mathiasson 1974; Dean 1978; Geldenhuys 1981a; Austin and Fredrickson 1986; Lovvorn and Barzen

1988; Vrtiska et al. 1997; Kear 2005) and the two life-history stages are not expected to overlap, because flightless moult severely impairs mobility and hence the ability of the adults to care for young.

The objectives of the study were to:

1. (a) Determine the timing of moult in Egyptian Goose, Spur-winged Goose, South African Shelduck, Yellow-billed Duck and Red-billed Teal at two sites in South Africa with opposite rainfall regimes (one summer, one winter).

   (b) Investigate how the timing of rainfall relates to the timing of breeding and moult in these five species.
2. Compare the relative timing of breeding and moult in southern hemisphere (southern African and Australian) waterfowl with northern hemisphere (European and North American) species.

Materials and Methods

Data collection in southern Africa

Timing of moult in southern African waterfowl was studied between February 2007 and April 2010 at Barberspan Nature Reserve (26º 33´ S, 25º 37´ E) in the North-West Province and Strandfontein Wastewater Treatment Works (34º 05´ S, 18º 32´ E) in the Western Cape Province, South Africa. Both sites are described in detail in Chapter 1.

Bi-monthly counts of waterfowl were made at both study areas for the duration of the study, with each sampling session lasting five days (see below). Thirteen accessible permanent sampling points (at least 500 m apart) were selected along the shoreline at each of the study sites and marked for repeated sampling and monitoring purposes. Sampling points were approached by an observer in a vehicle. Moulters (moulting birds that ran into the water and swam away using the characteristic ‘paddle fluttering’ wing action of flightless anatids - Dean 1978) and non-moulters were counted within a 150 m radius semi-circle from the sampling point using 10x42 binoculars. Counting at sampling sites was performed randomly over the five-day census period. Fifty-two counts were obtained for each study site per sampling interval, with each of the 13 sampling points being counted once a day, four times in five days, and at different times of the day. The total number of moulters and of non-moulters from all 52 counts was then computed to obtain a mean abundance per hectare for moulters and non-moulters of each species at each study site at each visit.

After the five days of counting, a further 5-8 days were dedicated to trapping. Blood samples were taken from moulting birds to determine their sex (Griffiths et al. 1998). This sampling process further validated the presence of moulting birds that were counted during the census period. However, trapping did not validate the ratios of moulters to non-moulters.

I recorded incidents of breeding at each site during the study period but my sample size was too small for all species other than Egyptian Geese because most waterfowl rarely bred at either study site. I therefore used breeding period records for Barberspan and Strandfontein (or from areas nearby in cases where breeding data were lacking) compiled from Hockey *et al*. (2005) to supplement my breeding data.

Decadal rainfall-estimate satellite images were downloaded for the period starting 1 June 2006 until 30 April 2010 from the United States Geological Survey (USGS) Africa Data Dissemination Service (2010) website (http://earlywarning.usgs.gov/fews/). These images were taken by NASA (National Aeronautics and Space Administration) from the NOAA (National Oceanic and Atmospheric Administration) satellites at 8 km resolution. I used GIS tools in ArcView 3.3 (ArcView Inc. 1992) to convert each of the 78 rainfall-estimate satellite images into a grid image. I used GIS tools to create buffer zones shape files of 250 km radius around each of the two study sites. In the case of Strandfontein, I used the South Africa outline map to clip off the sections of the buffer zone that overlapped with the ocean. I then used the clipped buffer-zone shape files to extract rainfall data from the grid images into spread sheets. Because my waterfowl census data were collected bimonthly, the matching rainfall values used in the analysis were averages of rainfall in the two months (mm; a total of six decadal values divided by two) prior to a sampling interval.

Northern vs southern hemisphere comparison

I used moult and breeding period records of waterfowl for Australia (New South Wales and Victoria), Europe and the Nearctic (Canada and the United States) compiled in Kear (2005), del Hoyo *et al*. (1992), BirdLife International (2012 - http://www.birdlife.org) and Marchant and Higgins (1990).

For the inter-hemisphere comparison I chose species that: (1) were similar in body size to each of the five southern African waterfowl species for which I determined timing of moult; (2) had available and reliable time of moult and breeding data; (3) belong to the Anatinae subfamily (except for Canada Goose *Branta canadensis*, Greylag Goose *Anser anser* and Greater White-fronted Goose *A. albifrons*); and (4) are either grazers and/or dabblers. The Canada Goose, Greylag Goose and Greater White-fronted Goose were included in the study because there were no native Anatinae subfamily species in the northern hemisphere that are of comparative size and body mass to Egyptian Goose and Spur-winged Goose. The study species are listed in Table 3 with their scientific names.

Statistical analysis

I used Spearman’s rho correlation test to test for associations of numbers of moulting southern African species at each site with rainfall. A one-way analysis of variance (ANOVA) was used to compare the time lags between peak breeding and peak moult periods in waterfowl from different regions (and sites in the case of southern Africa). All statistical analyses were tested at a 5% level of significance and carried out using the PASW 18 statistical program (SPSS Inc., Chicago IL 2009).

Table 1. Study species

Site	Common name	Species
Southern Africa	Spur-winged Goose	*Plectropterus gambensis*
	Egyptian Goose	*Alopochen aegyptiaca*
	South African Shelduck	*Tadorna cana*
	Yellow-Billed Duck	*Anas undulata*
	Red-Billed Teal	*A. erythrorhyncha*
Australia	Australian Shelduck	*Tadorna tadornoides*
	Australian Wood Duck	*Chenonetta jubata*
	Grey Teal	*A. gracilis*
	Chestnut Teal	*A. castanea*
	Mallard	*A. platyrhynchos*
	Pacific Black Duck	*A. superciliosa*
Europe	Common Shelduck	*Tadorna tadorna*
	Ruddy Shelduck	*T. ferruginea*
	Eurasian Wigeon	*A. penelope*
	Garganey	*A. querquedula*
	Greylag Goose	*Anser anser*
North America	Blue-winged Teal	*Anas discors*
	Common Teal	*A. crecca*
	Gadwall	*A. strepera*
	Northern Pintail	*A. acuta*
	Greater White-fronted Goose	*Anser albifrons*
	Canada Goose	*Branta canadensis*

Results

At both sites in South Africa, the abundance of moulting Spur-winged Geese, Egyptian Geese, Yellow-billed Ducks and Red-billed Ducks was significantly and inversely correlated with rainfall (Table 2). There was also a significant negative correlation between South African Shelduck moult and rainfall at Strandfontein, but not at Barberspan where shelducks moulted in the rainy season. There was a positive correlation between numbers of moulting South African Shelduck and rainfall at Barberspan (Table 2). Flocks of moulting waterfowl at both Barberspan and Strandfontein contained approximately equal proportions of males and females (Spur-winged Goose = 1:1.4, Egyptian Goose = 1:1, South African Shelduck = 1:1.1, Yellow-billed Duck = 1.2:1 and Red-billed Teal = 1:1).

Table 2. Spearman's rho correlation of moulting waterfowl numbers with rainfall. *Correlation is significant at the 0.05 level (2-tailed); **Correlation is significant at the 0.01 level (2-tailed) and; ^{ns} Correlation is not significant.

Site	Species	Correlation coefficient
Barberspan	Spur-winged Goose	-0.74 **
	Egyptian Goose	-0.65 **
	South African Shelduck	0.41 ^{ns}
	Yellow-billed Duck	-0.65 **
	Red-Billed Teal	-0.52 *
Strandfontein	Spur-winged Goose	-0.54 *
	Egyptian Goose	-0.62 *
	South African Shelduck	-0.67 **
	Yellow-billed Duck	-0.62 *
	Red-Billed Teal	-0.56 *

At both South African sites, peak moult occurred in the dry season for all species except for South African Shelducks: which moulted in the wet season at Barberspan. The amount of rainfall within a 250 km radius of Barberspan and Strandfontein did not differ during the last two months leading up to peak moult of the other four species, which was the driest period at both study sites (Fig. 1). This observation further confirms that southern African ducks (except for shelducks at Barberspan) were moulting at the height of the dry season. As a corollary, peak breeding of all species except shelduck occurred during the wet season (Table 3).

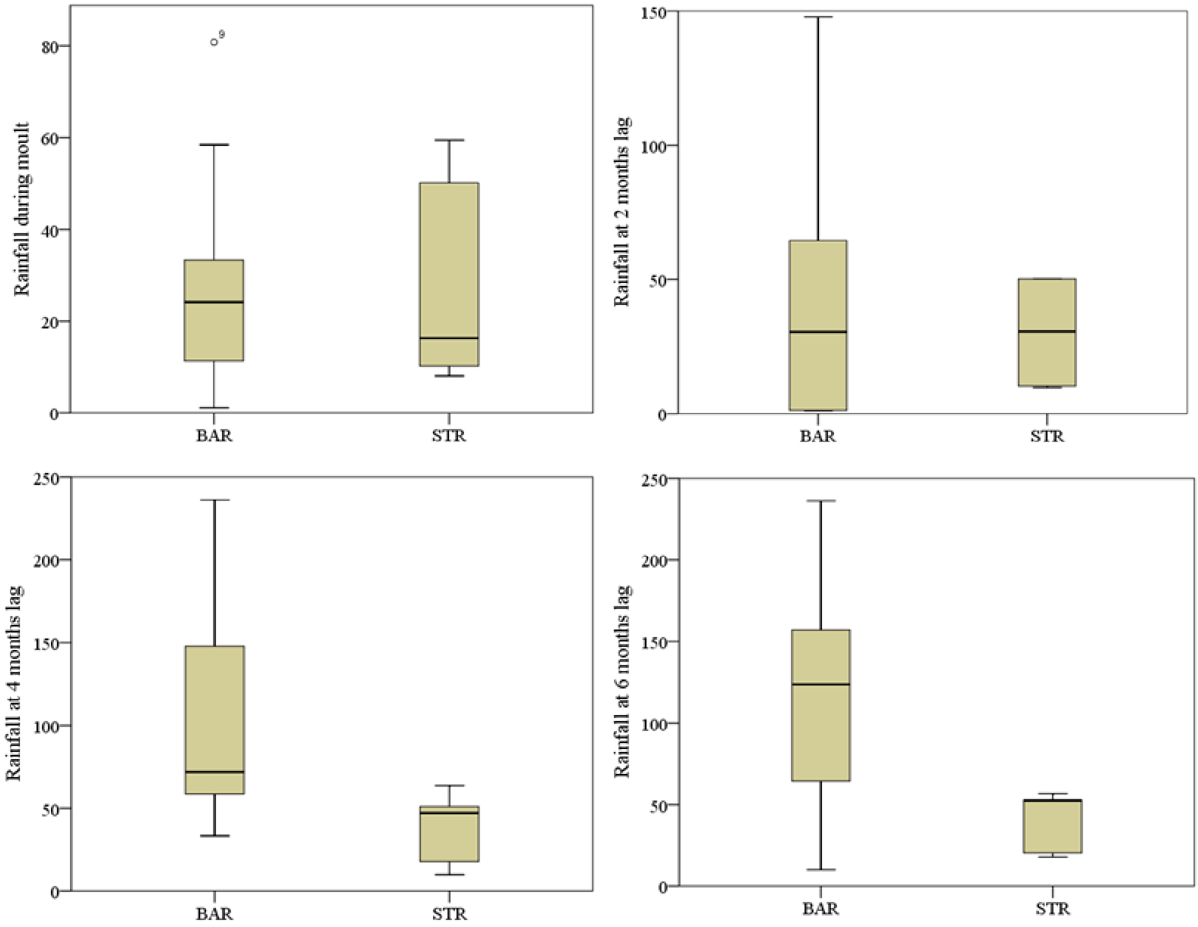

Figure 1. Box plots indicating the magnitude of rainfall at Barberspan and Strandfontein

during peak moult and during three (two months apart) lag periods preceding moult.

Table 3. Timing of moult and breeding of waterfowl in South Africa (Barberspan and Strandfontein), Australia (New South Wales and Victoria, below latitude 35ºS), Europe (above latitude 35ºN) and North America (above latitude 40ºN).

Key: M = Moult; B = Breeding. Combination labels: M(♂♀), males and females moult together at the same locality; M(♂)(♀), males and females moult at different localities. The label (B, M) indicates a population-level overlap between moult and breeding.

Site	Species	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Barberspan		Wet	Wet	Wet	Wet	Dry	Dry	Dry	Dry	Dry	Dry	Wet	Wet
	Spur-winged Goose					M(♂♀)	M(♂♀)	M(♂♀)			B	B	B
	Egyptian Goose				M(♂♀)	M(♂♀)	M(♂♀)			B	B	B	B
	South African Shelduck	M(♂♀)							B	B	B	B	M(♂♀)
	Yellow-billed Duck					M(♂♀)	M(♂♀)	M(♂♀)	M(♂♀)	B	B	B	B
	Red-billed Teal	B				M(♂♀)	M(♂♀)	M(♂♀)		B	B	B	B
Strandfontein		Dry	Dry	Dry	Dry	Wet	Wet	Wet	Wet	Wet	Wet	Dry	Dry
	Spur-winged Goose		M(♂♀)	M(♂♀)	M(♂♀)				B	B	B	B	B
	Egyptian Goose	M(♂♀)	M(♂♀)	M(♂♀)					B	B	B	B	M(♂♀)
	South African Shelduck	M(♂♀)							B	B	B	B	M(♂♀)
	Yellow-billed Duck		M(♂♀)	M(♂♀)	M(♂♀)	M(♂♀)			B	B	B	B	B
	Red-billed Teal	M (♂♀)	M (♂♀)	M (♂♀)	M(♂♀)				B	B	B	B	M(♂♀)
Australia		Dry	Dry	Dry	Dry	Wet	Wet	Wet	Wet	Wet	Wet	Dry	Dry
	Australian Shelduck						B	B	B	B	M (♂)	M (♂)	M (♀)
	Australian Wood Duck	M(♂♀)						B	B	B	B	M(♂♀)	M(♂♀)
	Grey Teal		M(♂♀)	M(♂♀)				B	B	B	B	B	B
	Chestnut Teal	M(♂♀)	M(♂♀)					B	B	B	B	B	
	Mallard	M(♂♀)	M(♂♀)						B	B	B		
	Pacific Black Duck	M(♂)	M(♂)(♀)	M(♀)	M(♀)		B	B	B	B	B	B	B, M(♂)
Europe		Cold	Cold	Cold	Warm	Warm	Warm	Warm	Warm	Warm	Warm	Warm	Cold
	Common Shelduck				B	B		M(♂)	M(♂)	M(♀)	M(♀)		
	Ruddy Shelduck				B	B	B, M(♂)	M(♂)					
	Eurasian Wigeon				B	B, M(♂)	B, M(♂)	M(♂)(♀)	M(♀)	M(♀)			
	Garganey				B	B	B, M(♂)	B, M(♂)	M(♂)(♀)	M(♀)			
	Greylag Goose				B	B	B, M(♂)	M(♂)(♀)					
North America		Cold	Cold	Cold	Warm	Warm	Warm	Warm	Warm	Warm	Warm	Warm	Cold
	Blue-winged Teal					B	B	B, M(♂)	M(♀)				
	Common Teal					B	B	B, M(♂)	M(♂)	M(♀)			
	Gadwall					B	B	B, M(♂)	M(♀)				
	Northern Pintail					B	B	B, M(♂)	M(♀)				
	G. White-fronted Goose					B	B	B, M(♂♀)	B, M(♂♀)				
	Canada Goose				B	B	B,M(♂♀)	B, M(♂♀)					

The general pattern of wet-season breeding and dry-season moult exhibited by southern African ducks was mirrored by waterfowl in Australia (Table 3). The sites from which the Australian data were drawn experience a very similar rainfall regime to that of Strandfontein, and the timing of breeding and moult between the two were closely synchronous, but with a tendency for Australian species to start breeding and moult slightly earlier in the year.

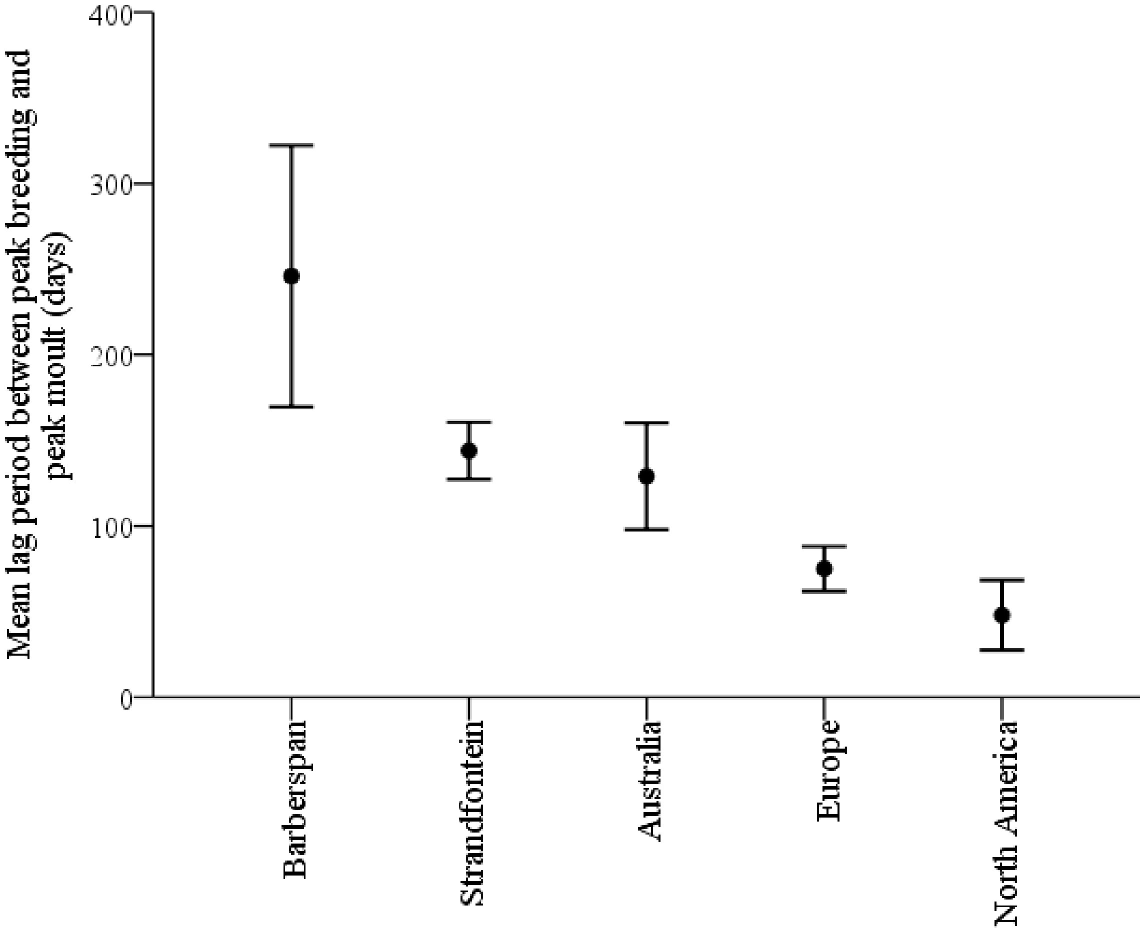

Figure 3. Mean lag periods (± 95% CI) between peak breeding and peak moult (days) for waterfowl in South Africa (Barberspan and Strandfontein), Australia (New South Wales and

Victoria, below latitude 35ºS), Europe (above latitude 35ºN) and North America (above latitude 40ºN).

There was a significant difference in time elapsed between peak breeding and peak moulting periods amongst waterfowl at different locations (ANOVA, F = 31.78, df (groups) = 4, $p > 0.0001$; Fig. 3). The lag period between peak breeding and peak moult for waterfowl at Barberspan was the longest (246 ± 27.5 days) and was significantly different ($p > 0.001$) from that of waterfowl at Strandfontein (144 ± 6.0 days [± 95% CI]), Australia (136 ± 11.9 days), Europe (75 ± 4.7 days) and North America (45 ± 6.7 days). Waterfowl at Strandfontein had a similar ($p = 0.995$) lag period between peak breeding and peak moult to waterfowl at south-east Australia. Waterfowl in Europe and North America had a very similar ($p = 0.999$) lag period between peak breeding and peak moult: these lag periods were significantly shorter ($p > 0.001$) than those of waterfowl in Australia and at Strandfontein (Table 3, Fig. 3).

Northern hemisphere waterfowl breed and moult during the warm season. Moult commenced soon after, and in some cases during breeding (Fig. 3). Male northern hemisphere ducks undertake migrations to moulting sites while the brood is still young, whereas females commence moult at breeding sites soon after the chicks fledge (del Hoyo et al. 1992). Among the northern geese, both male and female Greylag Geese, Greater White-fronted Geese and Canada Geese participate in rearing of goslings and both sexes moult at the breeding sites while goslings are still young (Kear 2005).

Discussion

The peak moult period of Spur-winged Geese, Egyptian Geese, Yellow-billed Ducks and Red-billed Teals at Barberspan confirms earlier studies (Shewell 1959; Siegfried 1967; Geldenhuys 1975), identifying a mid-winter (June-July) peak moult period in the north-east of South Africa (i.e. the middle of the dry season). The same four species also moulted in the middle of the dry season (mid-summer) at Strandfontein. In contrast to the other four species, South African Shelducks at Barberspan moulted in mid-summer (the height of the rainy season).

Moulting Red-billed Teals proved to be elusive and very few birds were recorded in moult at either site. Dean (1978) had a similar problem when counting moulting Red-billed Teals at several large pans in north-eastern South Africa (including Barberspan). He speculated that during wet years Red-billed Teal used smaller, seasonal pans (that were dry in low-rainfall years) to moult, hence their absence from larger pans. I suggest that the two study sites were not preferred moulting sites for Red-billed Teal.

My results also support the idea that breeding in most southern African waterfowl is triggered by rainfall (as proposed by Siegfried 1974) and that moult takes place after breeding is completed (Joensen 1973; Mathiasson 1974; Dean 1978; Geldenhuys 1981a; Austin and Fredrickson 1986; Lovvorn and Barzen 1988; Vrtiska *et al*. 1997; Kear 2005). In the south-western parts of South Africa (the winter-rainfall area) moult takes place soon after breeding, consistent with what is known about waterfowl elsewhere (Baldassarre and Bolen 2006). However, at Barberspan (in a summer-rainfall area) there was a lag period of at least three months between the end of breeding and the start of moult in Spur-winged Goose, Egyptian Goose, Yellow-billed Duck and Red-billed Teal (Table 3). It is not immediately clear why these birds delay moult for so long after breeding. Satellite telemetry results in Cumming *et al*. (2012) and Chapter 6 revealed that even though most Egyptian Geese and Red-billed Teal that moult at Barberspan and Lake Manyame (located in a summer-rainfall area in northern Zimbabwe - 17°49'S, 30°36'E) breed at distant locations (averaging 450 km and up to ≈1000 km away from the moulting site), these birds took at most a week to commute from their breeding sites to a moulting site. Although the lag period between breeding and moult differs significantly between Strandfontein and Barberspan, the timing of moult relative to peak rainfall was similar for both sites (Table 2). This implies that timing of breeding in the four study species (excluding shelducks), relative to peak rainfall, was flexible, but timing of moult was not. At both sites, however, the period of minimum rainfall during the year occurred in the two months prior to the onset of moult (Fig. 1). This provides some support that rainfall influences the timing of moult in southern African waterfowl above and beyond timing constraints imposed by the need to breed at a certain time of year. The timing of moult during the dry season in most southern African waterfowl and in Australian species is perhaps an adaptation to ensure that the birds become flightless and are restricted to permanent wetlands at the time when ephemeral pans have dried out and terrestrial landscapes offer the fewest breeding opportunities. In addition, by waiting until the height of the dry season to moult, birds could be avoiding the risk of moving to a pan which will itself dry out before they have completed moult.

The long lag between breeding and moult at Barberspan relative to other southern hemisphere sites also reflects the fact that birds in the summer-rainfall area start to breed earlier in the wet season than they do in either the winter-rainfall region of South African or in Australia (Table 3). A plausible explanation for this earlier breeding is that birds are trying to avoid the hottest time of year (Jan and Feb) for breeding to reduce thermal stress on incubating adults and young.

South African Shelducks seemingly are an exception to this generalisation, moulting in the middle of summer regardless of rainfall seasonality. Unlike the other species, timing of moult in South African Shelducks did not consistently correlate with rainfall, with all records of moulting birds being confined to the period December to January, peaking in December in both the summer- and winter-rainfall regions (Tables 2, 3). This narrow moulting window conforms to earlier studies of shelduck moult (Geldenhuys 1981a). Most South African Shelducks breed in the summer-rainfall region. In this region, dry-season breeding may have been selected because these birds nest in holes in the ground, often inside Aardvark *Orycteropus afer* tunnels (Hockey *et al*. 2005), and such nest sites may be prone to flooding during winter rains. The relatively new records of South African Shelducks breeding in the wet season in the Western Cape (Geldenhuys 1981b) suggest that their colonisation of this area may be comparatively recent, possibly in response to the proliferation of man-made wetlands throughout South Africa's arid west (Okes *et al*. 2008), and that they have not yet fully adjusted their breeding season in response to the different rainfall regime of the region (as has been the case with some other recent colonists of the winter-rainfall region – e.g. Curtis *et al*. 2007).

The sex-related partitioning in the timing of moult in northern-hemisphere waterfowl (Table 3) is due to the uni-parental care system in these species (Kear 2005). Males of most northern hemisphere ducks (Anatinae) do not participate in either incubation or brood care. Rather, during breeding, males gather in flocks and migrate to wetlands elsewhere to moult, leaving the females to care for the broods (del Hoyo *et al*. 1992). Females tend to moult at the breeding grounds while caring for the brood (del Hoyo *et al*. 1992), with the result that, overall, males moult at different times and in different locations to females. Among southern African waterfowl, by contrast, both sexes undertake moult migrations after the end of the breeding season and moult together. This is possible because in most southern African species, brood care is bi-parental (e.g. Egyptian Goose, Spur-winged Goose, South African Shelduck, White-faced Duck and Fulvous Duck *Dendrocygna bicolor* - Hockey *et al*. 2005).

Many waterfowl breeding at high northern latitudes are migratory (Kear 2005; Baldassarre and Bolen 2006). Many of the wetlands used for breeding have abundant food and water during the warm summer months but freeze over during winter: the few areas that remain unfrozen are insufficient to support the (increased) population after the breeding season (Baldassarre and Bolen 2006). The cold season is thus an environmental ‘stressor’ for waterfowl in the north, forcing them to complete both breeding and moult in rapid succession before the onset of the winter freeze (del Hoyo *et al*. 1992; Kear 2005; Baldassarre and Bolen 2006; Table 3). Fox (per comms) has also suggested that they breed during this time to avoid preditors.

It also appears that overlap between breeding and moult is a relatively common phenomenon amongst northern hemisphere waterfowl (Table 3). This overlap is most pronounced in those species with long periods of parental care (especially the Anserini species, e.g. Greater White-fronted Goose – Ely 1993). By comparison, at the lower latitude environments of the southern hemisphere in South Africa and Australia, winters are milder and the key environmental ‘stressor’ is the dry season (winter in the north east, and summer in the south west). Hence, most waterfowl in the south (except for hole-nesting South African Shelducks), breed during the rainy season, regardless of whether this falls in summer or winter.

I conclude that waterfowl in southern Africa (with the exception of South Africa Shelduck), south-east Australia, Europe and North America time their breeding period to coincide with peaks in the availability of both food and breeding sites (Owen and Cook 1977; Holmgren and Hedenström 1995). Northern hemisphere species moult during the same, narrow ‘window of plenty’, when temperatures are warm, and before food and aquatic habitats approach their winter minima (which most species avoid by migration). By contrast, southern hemisphere waterfowl delay the onset of moult until the dry season, opting to moult when both food and aquatic moulting habitats are in limited supply. I hypothesise, but cannot prove, that they do this to minimise the risk of starting moult at a water-body that may run dry before their moult is completed (which would leave the birds immobile and thus at the mercy of predators).

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CHAPTER 5

Moult-site fidelity of Egyptian Geese in South Africa

Abstract

Little is known about moult and moult-site fidelity of African waterfowl. Satellite telemetry and uniquely engraved colour-rings were used to study moult-site fidelity of Egyptian Geese marked at Barberspan and Strandfontein in South Africa. Twelve Egyptian Geese were tagged with satellite GPS PTT transmitters while moulting at Barberspan and Strandfontein during 2008 and 2009, and a further 527 were colour-ringed between June 2007 and March 2009. Bi-monthly point counts of Egyptian Geese and searches for colour-ringed birds were made at each study site from July 2007 until June 2010, resulting in 139 individual colour-ringed Geese being re-sighted during the study period. I explored Egyptian Geese dispersal distances away from the study sites and modelled moult-site fidelity from colour-ring return rates corrected for survivorship, sampling period efficiency and colour-ring detectability estimates. There were 12.0% and 29.7% colour-ring return rates for geese ringed at Barberspan and Strandfontein, respectively. Four of five moulting birds tagged at Barberspan with satellite transmitters returned to Barberspan to moult the following year. Two of these same birds moulted at Barberspan in the third year, while the other two birds moulted elsewhere. One out of seven Egyptian Geese satellite-tagged at Strandfontein returned to the same site to moult the following year. One bird moulted at a nearby pan (5 km away), two moulted at estuaries less than 30 km from Strandfontein and one bird moulted at a farm dam 56 km away. Two birds satellite-tagged at Strandfontein did not moult the following year and returned to moult at Strandfontein in the third year. The moult-site fidelity model suggested that a minimum of 16% of Barberspan birds and 51% of Strandfontein birds returned to the same wetland to moult the following year. The apparent low moult-site fidelity at Barberspan suggested by colour-ring resightings may reflect the small number of birds colour-ringed at the site.

Introduction

Fidelity to particular sites of use has been documented in many species of far-ranging waterfowl (e.g. Hestbeck *et al*. 1991; Seymour 1991; Wilson *et al*. 1991; Anderson *et al*. 1992; Johnson 1996; Lindberg and Sedinger 1997; Robertson and Cooke 1999). In general, site fidelity in birds is often associated with higher survivorship and lifetime breeding success (Lindberg and Sedinger 1997; Robertson and Cooke 1999). The hypothesised benefit of such site fidelity is that individuals become familiar with particular sites and the resources they provide, and are not continually confronted with novel environments that require 'exploration' (Lindberg and Sedinger 1997; Robertson and Cooke 1999). However, waterfowl that exhibit high site fidelity and also aggregate in large numbers for a particular life-history stage such as flight-feather moult are potentially at risk should the preferred site be impacted by habitat loss or degradation (Anderson *et al*. 1992). Aggregation also places such birds at elevated risk from disease outbreaks (Tracey *et al*. 2004). Therefore, understanding the degree of site fidelity and patterns of movement among waterfowl is important for predicting potential population-level effects resulting from habitat change or disease.

Most waterfowl exhibit fidelity to breeding (e.g. Seymour 1991; Milstein 1993) and non-breeding sites (Robertson and Cooke 1999), but less is known about fidelity to moulting sites (Anderson *et al*. 1992; Bollinger and Derksen 1996; Flint *et al*. 2000). To date, all studies of moult-site fidelity among waterfowl come from migratory northern hemisphere species. For example, Black Brant Geese *Branta bernicla nigricans* have 95% moult-site fidelity (Bollinger and Derksen 1996), Gadwall *Anas strepera* 67% (Szymczak and Rexstad 1991) and American Black Ducks *A. rubripes* 52% (Bowman and Brown 1992). In Africa, it is well known that over 70% of the South African Shelduck *Tadorna cana* population moults at only

23 locations in South Africa (Geldenhuys 1981) but individual fidelity to moult-site is unknown.

The objective of this chapter was to determine inter-annual moult-site fidelity of Egyptian Geese *Alopochen aegyptiaca* at two geographically separated sites in South Africa, Barberspan Nature Reserve in the North West Province and Strandfontein Wastewater Treatment Works in the Western Cape Province. In particular, I aimed to estimate the proportion of Egyptian Geese that return to moult at the same site in the following year and in consecutive years thereafter. I modelled moult-site fidelity from colour-ring return rates correcting for survivorship, sampling period efficiency and colour-ring detectability estimates. I also explored the dispersal patterns and distances moved by marked Egyptian Geese outside the two study sites.

Egyptian Geese historically inhabited semi-arid areas with few and scattered perennial water bodies (Milstein 1993). I therefore hypothesised that there was an evolutionary pressure for high moult-site fidelity to the few historical, perennial water bodies where moult was possible. I also hypothesised that this signal would be conserved and that Egyptian Geese, like South African Shelducks (the only other Tadorninae species in southern Africa - Milstein 1993) will display high moult-site fidelity to historically perennial water bodies (Geldenhuys 1981), despite the present-day southern African environment that has numerous man-made perennial water bodies.

I also tested for differences in moult-site fidelity between the two study sites. The ‘plasticity’ hypothesis predicts that levels of moult-site fidelity, in Egyptian Geese are related to the birds’ present-day environment, in particular the availability of suitable moulting water bodies. Egyptian Geese that inhabit areas with abundant perennial water bodies will moult within the same region but exhibit low moult-site fidelity because the modern landscape presents the birds with a diversity of perennially suitable moulting habitats (Geldenhuys

1975). I therefore predicted that Strandfontein birds would exhibit low moult-site fidelity compared to Barberspan birds, because the south-western region has a higher density of man-made perennial water bodies than the semi-arid north-eastern region of South Africa (where Barberspan is located).

Methods

I used satellite telemetry and uniquely engraved colour leg rings to investigate moult-site fidelity of Egyptian Geese tagged at two study sites in South Africa, Barberspan Nature Reserve (26º 33´ S, 25º 37´ E) and Strandfontein Wastewater Treatment Works (34º 05´ S, 18º 32´ E). The two sites are approximately 1000 km apart, with Barberspan receiving variable but predominantly summer rainfall and Strandfontein receiving rain in winter. The region surrounding Barberspan has fewer perennial water bodies compared to the region surrounding Strandfontein. Both study sites are described in Chapters 1 and 2. Egyptian Geese have been recorded as moving between the two sites (Underhill *et al*. 1999) although such movements appear to be rare. Egyptian Geese were caught and ringed at Barberspan and Strandfontein between May 2007 and April 2009.

Satellite telemetry

Twenty-two moulting Egyptian Geese at the two study sites were tagged with 30 g, solar-powered GPS PTT satellite transmitters manufactured by Microwave Telemetry, Columbia, USA. Nine birds were tagged at Barberspan (six in June 2008 and three in June 2009) and 13 birds were tagged at Strandfontein (eight in January 2008 and five in January 2009). Transmitters were attached to birds using a backpack harness made of Teflon ribbon, with loops in front of and behind the wings attached in a cross design and sewn together at a single point over the sternum. The transmitter attachment method and design are described in more detail in Cumming and Ndlovu (2011, see Appendix 1). Transmitters were set to capture GPS location data every two hours and to transmit data to the satellite every three days. Only 12 transmitters (five from Barberspan and seven from Strandfontein) lasted for more than one year, i.e. into the next moult season: only data from these 12 birds were considered.

Colour-rings and counts

A sample of moulting Egyptian Geese was captured and ringed during peak moult period using maize-baited, walk-in traps placed near the water’s edge. A total of 527 Egyptian Geese were marked, 100 at Barberspan and 427 at Strandfontein. Each goose was ringed on the left leg with a SAFRING metal-ring and on the right leg with a 16 mm (inside diameter) by 28 mm (high) acrylic colour ring supplied by Protouch Engraving Canada (www.birdband.com); each colour ring was engraved with a unique three-letter code. Colour-rings weighed 3.7 g and each metal ring weighed 5.1 g. Only birds with adult plumage were ringed. The lightest Egyptian Goose ringed had a body mass of 975 g, therefore the combined mass of 8.8 g for both rings made up 0.9% (at most) of the bird’s body mass. The supplier claimed that the colour rings were expected to degrade and fall off the birds only after about five years, i.e. beyond the duration of this study. In addition to colour ringing, information on flight-feather moult, body mass and biometric data (tarsus, head, wing and bill length) were collected.

Point counts of Egyptian Geese and searches for colour-ringed birds were carried out bimonthly and after a complete moult cycle (i.e., during the next peak moult period) at each study site from July 2007 until June 2010. Sampling was undertaken during a 10-day period that included five days of counts followed by five days of trapping. Trapping was carried out in addition to searches to maximise colour-ring returns (Barker 1999). Egyptian Geese were counted at 13 permanent points at each site to estimate their abundance. Birds were counted within a semi-circle of 150 m radius from the sampling point. Each point was counted four times (once a day, at a different time of the day to capture daily variation) and counts were spread across a five-day period. The total number of Egyptian Geese from all 52 counts for each study site was divided by four (to get a per-count average) and this value was used to estimate population numbers at searched locations. Searches for colour-ringed birds were simultaneously carried out during the counting and trapping periods. Colour-ring searches were undertaken on average for six hours a day at each site using 10x42 binoculars and a 20x60 telescope. It was not possible to check all individuals for colour-rings because some birds entered the water when an observer approached. Colour-ring return rate, recorded as a percentage, was determined as the proportion of colour-ringed birds from the previous year that were re-sighted or caught in the following years.

Due to time and logistical constrains, scans for colour rings at the North West Province were limited to Barberspan nature reserve. The study also relied on the public for returns from outside the reserve. In the area surrounding Strandfontein, monthly searches for colour-rings were conducted at pans on seven golf courses, and several public parks, sports fields and urban dams. A pamphlet requesting re-sightings of colour-ringed Egyptian Geese from the public was posted online and some posters were placed at golf courses and on public notice boards.

Moult-site fidelity model

Moult-site fidelity is measured by the return rate of marked individuals as a proportion of the marked individuals in the local population. Estimates of these parameters are influenced by ecological processes and sources of sampling bias. The most important of these include: (1) survivorship (birds with rings die and thus leave the population); (2) recruitment (unringed juvenile birds enter the population); (3) movement (birds are added or removed from the population by dispersal); (4) sampling efficiency (the match between sampling periods and the periods during which individuals are present at the site); and (5) detectability (rings are imperfectly detected by an observer).

I developed a simple population model to estimate the level of moult-site fidelity for any given colour-ring return rate correcting for survivorship, sampling period efficiency and colour-ring detectability. The model is based on principles derived from the Cormack-Jolly-Seber recapture model (Cormack 1964). I did not use the program MARK (White and Burnham 1999) to analyse the data because I had a relatively small sample size of marked birds (n = 100 for Barberspan and n = 427 for Strandfontein) and low colour-ring returns.

The annual survival rate of Egyptian Geese in South Africa is not known (Milstein 1993; Lensink 1998). For the purposes of this model I used the regression equation of Krementz *et al*. (1997) to estimate the average annual survival rate for Egyptian Geese:

$$\text{Survivorship } (s) = 0.103\text{Mass} + 0.655$$

This regression equation is based on an established relationship between average annual adult survival rate and mean body mass derived from 40 species of swans, geese and ducks sampled from different parts of the world (Krementz *et al*. 1997). The average body mass of Egyptian Geese was 2.2 kg (Chapter 2) giving an estimated annual survival rate of 88%. This figure is similar to Lensink's (1998) estimates of 85% for Egyptian Geese in the Netherlands. However, because Egyptian Geese in South Africa are occasionally shot as crop pests, there is a possibility that the true annual survival rate may be slightly lower. I therefore also ran the model with a lower survival rate of 60%, the lowest survival rate calculated for other large ducks and small geese (Cramp and Simmons 1977).

Recruitment is the annual rate at which young Egyptian Geese are added to the local population by reproduction. However, it is modified to include both reproduction input and mortality. Egyptian Geese populations in the Western Cape are increasing (Mangnall and Crowe 2002) because annual reproductive success exceeds annual mortality (or because of immigration). Recruitment of individuals (without colour-rings) into the local population will decrease detectability of individuals with colour rings.

Dispersal of ringed birds to moult at other locations will be reflected as a reduction of moult-site fidelity, even though I could not distinguish dispersal from mortality in this study. Furthermore, movement of birds from other regions to moult at a study site will decrease detectability and lower proportional colour-ring returns. Movement of non-ringed individuals from other regions into the local population and from the local population to outside regions cannot be directly estimated or distinguished from reproductive recruitment and mortality, respectively. This is often overcome by assuming that emigration equals immigration, which renders it impossible to quantify the relative contributions of the two dispersal processes to the dynamics of the population. For the purposes of this model, I also had to make the assumption that emigration equals immigration.

Sampling efficiency was measured as the proportion of the surviving ringed population that was present and moulting during the sampling period (*t*+1). The mean moult-cycle period of Egyptian Geese in South Africa is 366 days (SD = 73, *n* = 30; Milstein 1993). Moult cycle duration of individual adult Egyptian Geese has been reported to be normally distributed with no significant differences between sexes (Milstein 1993). A typical peak moult sampling period in my study lasted 10 days and was carried out 361-371 days after the previous sampling/tagging period (Fig. 1). The model was therefore based on the assumptions that: (1) Egyptian Geese are flightless and restricted to a moult-site for at least 28.5 days; (2) I sampled at or close to the peak in abundance; and (3) moult-cycle duration in colour-ringed individual geese is normally distributed and the sampling protocol thus covered a maximum of 62.66% (Fig. 1) of the surviving sample population during the sampling period (*t*+1).

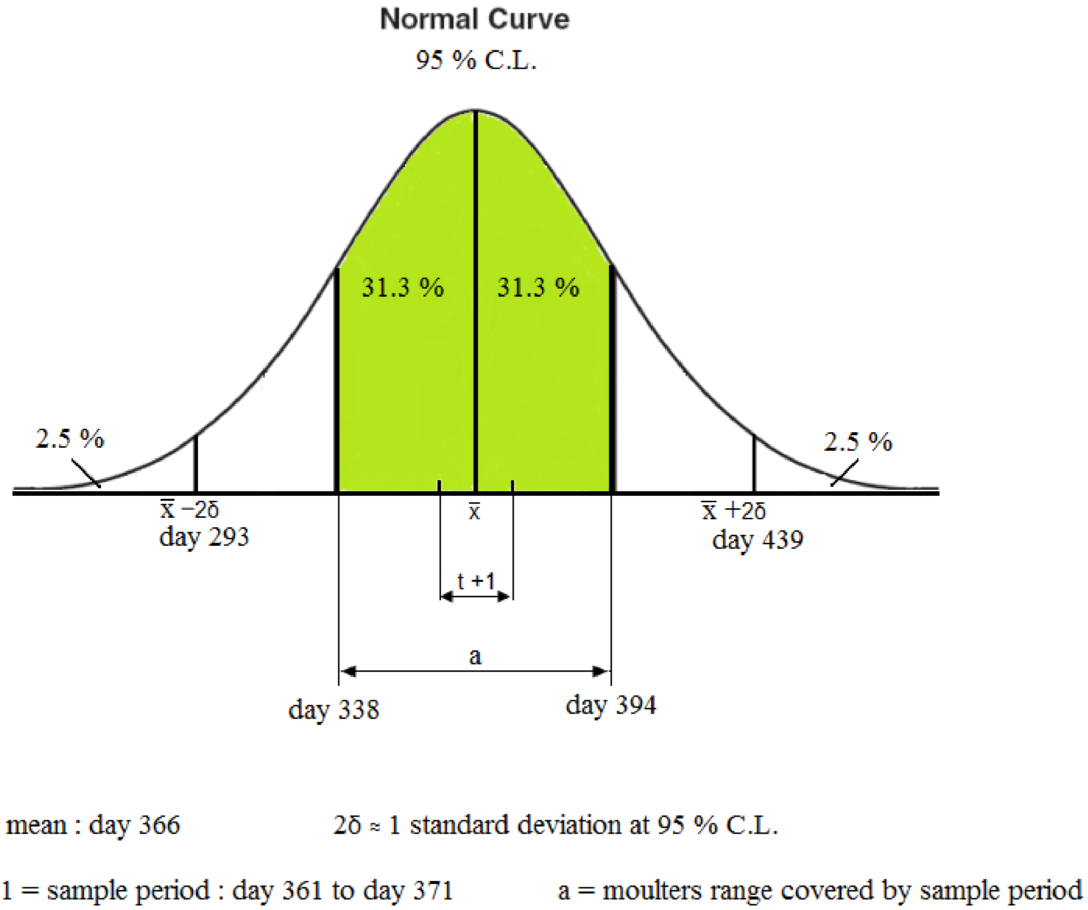

Figure 1. Normal distribution curve of moult-cycle duration of individual adult Egyptian Geese and the proportion of the sample population (shaded area) covered by the sampling protocol.

Detectability is the probability of re-locating all colour rings present at a site during the sampling period. It is inevitable that some colour rings will go undetected. A number of factors contribute to this problem. In particular, (1) moulting geese spend much of their time swimming, making it is impossible to observe leg-mounted colour rings; (2) high densities of moulting birds makes individual legs harder to see as birds are concealed behind one another; and (3) rain, cloudiness and observation distance affect visibility. It is therefore difficult to measure detectability and current methods of quantifying detectability are questionable (Gibbons and Gregory 2006). I thus assumed that the probability of detecting colour-rings was influenced primarily by the size of the local Egyptian Goose population present at the site, with the probability of individual detection decreasing as population size increases (the half normal detection function - Burnham and Anderson 1976; Zhang 2011). I assumed that the detection function (d) is the power of the corrected colour-ring returns and is dependent on the local population size (n). I assumed from the bird counts (Chapter 4) that the maximum carrying capacity at either site would be 20 000 Egyptian Geese. If there is one colour ringed bird at the site, the probability of detecting that bird is one ($d \approx 1$). However if there is only one colour-ringed bird when the population is at its maximum (20 000 birds) then the probability of detecting that bird tends to zero. As the population increases to estimated maximum carrying capacity, so the ability to detect colour ringed birds is correspondingly reduced. The probability of not detecting a colour ringed bird for any given local population size becomes $n/20\,000 = (5 \times 10^{-3})\, n$. Therefore d has the form:

$$d = 1 - (5 \times 10^{-3})\, n, \text{ given that } 20\,000 \geq n \geq 1$$

Where 5×10^{-3} is the approximate detectability constant, and n is the total number of birds marked with colour-rings. This equation assumes detectability to be high ($d \approx 1$) when local population size is less than 1 000 individuals.

To approximate the relationship between resightings and moult-site fidelity, I developed a model to capture the main components of the system. The model for moult-site fidelity (F) had the form:

$$F = (a/(bes))^{d}$$

Where 'a' is the total number of re-sighted colour-rings, b is the total number of birds marked with colour-rings, e is the sampling efficiency, d is detectability and s is survivorship.

To keep this model simple, I made the following assumptions:

1. Every ringed bird present in the population during the sampling period had the same probability of being detected;
2. Every ringed bird at time t has the same probability of survival until the next year's sampling period at t+1;
3. None of the colour rings were lost from marked birds and all resightings were recorded correctly during the study;
4. The number of dispersers from the population equaled that of immigrants into the population, meaning that no additional correction for dispersal is needed.

Statistical analysis

I used a one-way analysis of variance (ANOVA) to compare moult-site fidelity indicated by colour-rings between the two study sites for each year. Pearson correlations were used to test for the relationship between colour-rings sighted and the number of geese present at a site. All statistics were tested at the 5% level of significance and all analyses were carried out using the package IBM SPSS 19 (SPSS Inc.2010).

Results

Satellite telemetry

Four of the five birds satellite-tagged at Barberspan returned to Barberspan to moult the following year. Two of those same four birds moulted at Barberspan in the third year, while the other two birds moulted at two other sites <300 km away (Table 1). Between moults, three Barberspan birds dispersed maximum distances of over 600 km and the other two birds moved maximum distances of 409 km and 285 km from Barberspan. Of the seven birds satellite-tagged at Strandfontein, only one returned to moult there the following year. One bird moulted at a nearby pan (5 km away), two moulted at estuaries less than 30 km away, and one moulted at a farm dam 56 km away. Two birds satellite-tagged at Strandfontein did not moult the following year and returned to moult at Strandfontein in the third year. None of the seven Strandfontein-tagged birds travelled more than 132 km from the moult site. Four birds dispersed maximum distances of 103-132 km, while the remainder travelled less than 90 km from Strandfontein (Chapter 6). There were no apparent sex differences in moult-site fidelity (Table 1).

Table 1. Moult-site locations of 12 satellite-tagged Egyptian Geese. Column heading PTT 77- is transmitter identity. ** Did not moult that particular year.

PTT 77-	Sex	1st year moult-site	2nd year moult site	Distance from 1st moult (km)	3rd year moult site	Distance from 2nd moult (km)
127	♂	Barberspan	Motheo Farm1	330	Motheo Farm2	6
128	♀	Barberspan	Barberspan	0	Barberspan	0
130	♂	Barberspan	Barberspan	0	Barberspan	0
132	♀	Barberspan	Barberspan	0	Central Karoo	662
122.2	♂	Barberspan	Barberspan	0	-	-
094	♀	Strandfontein	Cape Point	20	-	-
095	♀	Strandfontein	Sandvlei	5	-	-
133	♂	Strandfontein	**	-	Strandfontein	0
134	♀	Strandfontein	**	-	Strandfontein	0
135	♂	Strandfontein	Paarl	56	-	-
118.1	♂	Strandfontein	Strandfontein	0	-	-
118.2	♀	Strandfontein	Rietvlei	27	-	-

Colour-rings

Of the 527 colour-ringed Egyptian Geese, 139 (26.4%) were subsequently resighted at least once during the study period. Of the 100 birds colour-ringed at Barberspan, there were 11 resightings on site (Table 2) and one bird (ringed in February 2009) was shot 14 months later near Hartebeespoort Dam (25°43'S, 27°49'E), 243 km away. Of the 427 birds ringed at Strandfontein, 169 (29.7%) were resighted, 158 of these being at the moulting site (Table 2).

Overall, one hundred and fifty-four birds ringed at Strandfontein while moulting in summer returned to the same site to moult the following year (Table 2) but none of these birds were resighted at Strandfontein during the non-moulting interim. Nineteen birds that were colour-ringed while moulting in December 2007 subsequently returned to moult in January 2008, again in January 2009 and one returned again in January 2010. Twelve birds that were colour-ringed while moulting in January 2008 subsequently returned to moult at Strandfontein in January 2009 and again in January 2010.

Of the birds ringed at Strandfontein, 35 were resighted elsewhere within the Western Cape. Excluding birds resighted at the moulting site, nine were resighted within a 10 km radius, 25 were resighted at distances of 10-50 km, and one was shot 129 km from Strandfontein. Eighteen birds were resighted at golf courses, two at the University of Cape Town campus and 14 in suburban parks. Only two birds were recorded from areas in agricultural lands: both of these were shot, suggesting that many more birds may move to agricultural areas than were reflected in colour-ring returns. One bird was recorded approximately 20 km to the south, near Cape Point, where it subsequently remained to moult.

Moult-site fidelity model

Using the moult-site fidelity model, and assuming survivorship of 88%, colour-ring resightings indicated a low moult-site fidelity for Barberspan (mean 16.97 ± 13.13%, n = 3 years; Table 3) and moderate moult-site fidelity for Strandfontein (mean 51.50 ± 4.93%, n = 3 years; Table 3). Assuming a lower survivorship of 60%, the moult-site fidelity for Barberspan remained at less than 25% (mean 23.70 ± 18.17%, n = 3; Table 3), while moult-site fidelity for Strandfontein approached 75% (mean 74.77 ± 6.92%, n = 3; Table 3). However, inter-annual variability was high and moult-site fidelity for the two sites did not differ significantly (88% survivorship: F = 6.067, P = 0.069, n = 6; 60% survivorship: F =

6.901, P = 0.058, n = 6; Table 3). Moult-site fidelity peaked at Barberspan in 2009 (42.8% and 59.4% for 88% and 60% survivorship, respectively) and at Strandfontein in 2008 (60.4% and 87.2% for 88% and 60% survivorship, respectively; Table 3).

Table 2. Return matrix for Egyptian Geese colour-ringed at Barberspan and Strandfontein.

Study site	Year	Numbers ringed	Numbers of colour-rings returned		
			2008	2009	2010
Barberspan	2007	7	0	0	0
	2008	44	-	9	0
	2009	49	-	-	2
	Total	**100**	**0**	**9**	**2**
Strandfontein	2007	141	46	19	1
	2008	210	-	58	12
	2009	76	-	-	18
	Total	**427**	**46**	**77**	**31**

Table 3. Moult-site fidelity and detectability of Egyptian Geese colour-ringed at Barberspan and Strandfontein. *s = annual survivorship.

Study Site	Year	Ring returns	Birds counted	Detectability	Moult-site fidelity %	
					*s = 88%	s = 60%
Barberspan	2008	0	1402	0.9299	0	0
	2009	9	2876	0.8562	42.8	59.4
	2010	2	706	0.9647	8.1	11.7
Strandfontein	2008	46	737	0.9631	60.4	87.2
	2009	58	308	0.9846	50.7	73.8
	2010	18	203	0.9898	43.4	63.3

The number of colour-ringed geese re-sighted at Barberspan was not significantly correlated with the total numbers of geese counted (r = 0.31, n = 18 sampling intervals, P = 0.21). Whereas, resightings at Strandfontein were significantly correlated with the total number of birds counted (r = 0.79, n = 18 sampling intervals, P < 0.001), possibly due to the larger number of birds ringed at this site.

Discussion

Satellite telemetry at both sites and colour-ring returns at Strandfontein indicated moderately high moult-site fidelity (within a 5 km radius of the initial moult site) for Egyptian Geese (Tables 1, 2, 3). By contrast, colour-ring returns at Barberspan suggested low moult-site fidelity, but this estimate was probably affected by (1) the small number of colour-ringed birds relative to the large number of unmarked geese present during peak moult (Tables 2, 3); and 2) the larger area of Barberspan compared to Strandfontein (Chapter 1). However, assuming a lower survivorship (60%) at Barberspan, colour-ring returns suggested moderately high moult-site fidelity in 2008 of at least 59.4% (Table 3). There was uncertainty in actual survivorship and whether it was the same for both sites. Therefore actual moult-site fidelity at each site is assumed to lie within the range modelled for 88% and 60% survivorship (Table 3).

None of the colour-ringed or satellite-tagged geese remained at either study site year round. During the winters of 2008 and 2009 (June to August), when breeding activity peaks in the Western Cape (Little *et al*. 1995), only three unmarked, resident pairs of Egyptian Geese remained at Strandfontein - compared with peak counts of 737 and 308 birds respectively during the summer moulting seasons. This conforms to the waterfowl-moult-migration hypothesis, of Salomonsen (1968), in this case that some permanent water pans that offer refuge from predators, even where foraging conditions are poor, are inhabited by ducks only when moulting. Strandfontein and Barberspan both previously have been documented as preferred waterfowl moulting sites (Shewell 1959; Taylor *et al*. 1999; Kalejta-Summers *et al*. 2001). Taken together, these observations suggest moderately high moult-site fidelity with the majority of birds visiting these sites mainly to moult (Tables 1, 2, 3).

Results obtained from the two methods used to assess moult-site fidelity presented an opportunity to test the accuracy of each method and what can be inferred from them about the biology of Egyptian Geese. Although colour rings are more visible than metal rings, they are invariably under-recorded due to sampling protocols and detectability: moult-site fidelity is, therefore, probably underestimated. Moult-site fidelity from colour-ring returns must be corrected for survivorship, sampling protocol and detectability bias. GPS satellite telemetry on the other hand is more accurate, but due to high costs typically translates to smaller sample sizes. Conclusions about Egyptian Geese moult-site fidelity from satellite telemetry results are therefore considered most reliable.

Telemetry data suggest that moult-site fidelity was higher at Barberspan than at Strandfontein (the opposite pattern to that indicated by ring resightings). Four of five satellite-tagged birds returned to moult at Barberspan in the second moult season and two of the four birds still transmitting returned in the third year (Table 4). In between the moult seasons these four geese breed at areas away from Barberspan, spending at least 200 days at distant sites, 400-600 km away. Birds tagged at Barberspan ranged over a large and semi-arid area consisting of farmlands in savanna and grasslands biomes (Chapter 5). Most of the ephemeral wetlands north-east of southern Africa, that are occupied by Geese during breeding season, dry out during winter, forcing birds to move to Barberspan and moult (Dean 1978). Barberspan is perhaps the only large pan in the area with sufficient water for a safe moulting habitat. Interestingly, when returning to Barberspan, satellite-tagged birds flew hundreds of kilometres, mainly at night, in an approximately straight line (Chapter 5).

In contrast to Barberspan birds, birds tagged at Strandfontein ranged over comparatively small areas, remaining within the lowland fynbos of the Cape Flats that lie below the escarpment of the Hottentot Holland mountain ranges, approximately 80 km east of Strandfontein (Chapter 5). Unlike the semi-arid, north-eastern parts of South Africa, the Cape area has at least 20 golf courses (with short, palatable grass available year round) and several impoundments that hold water all year round. Such a landscape presents Egyptian Geese with a diversity of perennially suitable foraging and breeding habitats, allowing the birds to be regionally resident. It is not clear what proportion of these abundant wetlands within the Western Cape lowlands are suitable for moult, but telemetry suggests that Egyptian Geese in this region favour moulting sites at wetlands close the coast that experience low levels of disturbance. Telemetry results thus suggest that levels of moult-site fidelity in Egyptian Geese (highest in the arid north east) are to some extent also influenced by the birds' present-day environment, even though they still carry a strong, historical signal.

Although historical ringing records indicate occasional movement of Egyptian Geese between Barberspan and Strandfontein (Underhill *et al*. 1999), no such movements were recorded in this study, by either colour-ringed or satellite-tagged birds. Failure to detect these movements in this study may be a consequence of small sample size (n = 527, versus a sample of 7218 metal-banded birds in Underhill *et al*. 1999), but support the conclusion that such movements are rare.

In summary, it is clear that most birds that visited Barberspan and Strandfontein did so primarily to moult. This is analogous to moult migrations undertaken by several waterfowl species in the northern hemisphere (e.g. Szymczak and Rexstad 1991; Bowman and Brown 1992). Given that flightless, moulting geese may be vulnerable to starvation and predation (Sjöberg 1986), the advantages of moult-site fidelity include knowledge of food resources, predator densities and habits. Individuals faithful to moult sites may use their local knowledge to avoid predators and exploit food resources, thereby increasing their survival (Lindberg and Sedinger 1997; Robertson and Cooke 1999).

Before the advent of urbanisation and irrigation, it is likely that movements of all Egyptian Geese (and other waterfowl species) in southern Africa were more extensive and probably more nomadic than they are today. Crop agriculture and urban parklands have increased the predictability of the food supply, and impoundments and wastewater treatment plants have increased the availability of permanent water bodies for moulting (Geldenhuys 1975). Both of these factors are likely to have modified waterfowl movements (Okes *et al*. 2008; Cumming *et al*. 2012) and may, at least in the winter-rainfall area, have reduced the need for Egyptian Geese to moult at the large perennial pans that were available historically. However, despite the context of the present-day environment, Egyptian Geese at both sites do exhibit moderately high moult-site fidelity. Perhaps the birds' present-day movements still carry a historical signal, reflecting a time when food resources were more scattered and perennially standing water was much scarcer, meaning moult was possible only at a few locations.

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CHAPTER 6

Life history and location dominate the daily movement patterns of Egyptian Geese in South Africa

Abstract

Unravelling patterns of movement at different spatial and temporal scales is essential for our understanding of the ecology, life history, behaviour and conservation of Afrotropical waterfowl. In this chapter I document the use of satellite telemetry to record the daily movements of Egyptian Geese. I used general additive mixed models to investigate distances moved by geese at different times of the day, correcting for gender, site, season and individual. I compared distances moved by geese in the winter-rainfall, south-west region of South Africa with movements in a semi-arid, summer rainfall region of northern South Africa. In the winter-rainfall region, water bodies are less patchy in space and more predictable over time than in the summer-rainfall region. Of the 21 transmitters that were deployed, 18 gave reliable results. Moulting Egyptian Geese all remained at the same wetland during moult and their daily movement patterns were similar at both moulting sites. Moulting birds displayed crepuscular patterns of activity and nocturnal retreats to safety. At the end of moult, Egyptian Geese from both sites dispersed away from their moulting sites. Most of the longest daily distances travelled by individuals occurred within the first 10 days after completing moult and just before the moult cycle was due to restart, providing compelling evidence for Egyptian Geese undertaking moult migrations. The pattern of daily movement was strongly skewed, with over 70% of flights being short (<10 km) and longer flights mainly occurring at night (88%). There were considerable differences in patterns of dispersal between birds at the summer-rainfall site at Barberspan and the winter-rainfall site at Strandfontein. Barberspan birds undertook long-distance movements between wetlands whereas Strandfontein birds appeared to move randomly and over short distances, characteristic of nomadism. Proximate cues for movement by Strandfontein birds function at the local landscape level, whilst birds from Barberspan rely mainly on memory when travelling long distances.

Introduction

Movement at different spatio-temporal scales allows mobile animals like birds to satisfy their resource requirements and adapt to spatially and temporally changing environments. Indeed, movements of individuals are vital components of many ecological and evolutionary processes and are integral to the maintenance of local populations, meta-populations, communities, and ecosystems (Levin 1974; Pyke 1984; Morales and Ellner 2002; Fauchald and Tveraa 2006).

Movement is fundamental to individual and population dynamics. For example, movements of waterfowl in the short term enable individuals to search for food, mates and escape predators, whereas in the long term, movements are important in avoiding interspecific competition, and locating breeding and moult sites. These movements arise from fine-scale behavioural decisions made by individuals in response to environmental heterogeneity at multiple spatial and temporal scales (Zollner and Lima 1999). Waterfowl decisions about movement are therefore linked intimately to survival, reproduction and moult. Most studies of movement focus on a particular biological context, such as dispersal (e.g. Oatley and Prŷs-Jones 1986; Underhill *et al*. 1999), foraging (e.g. Petrie and Rogers 1997) or migration movements (e.g. Gudmondsson *et al*. 1995; Dugger 1997; Miller *et al*. 2005), Despite their relevance to life-history strategies, there are few fine-scale studies that follow movements of individual waterfowl over long periods of time (Nathan *et al*. 2008; Webster *et al*. 2002), although daily movements of Grey Teals *Anas gracilis* have been followed at different times of year in Australia (Roshier et al. 2006).

Until recently, our understanding of waterfowl movement patterns and habitat use was limited largely by our inability to track individuals for long periods of time over extensive geographic areas (Lindberg and Walker 2007). However, since the inception of small, lightweight satellite transmitters, movement patterns of waterfowl in the northern hemisphere increasingly are being explored (e.g. Gudmondsson *et al*. 1995; Dugger 1997; Miller *et al*. 2005). Most of these studies, however, are of long-distance migrants and few studies focus on Afrotropical waterfowl with limited dispersal movements (e.g. Petrie and Rogers 1997). Petrie and Rogers (1997), in the first telemetry study on waterfowl in Africa, described the movement patterns of White-faced Ducks *Dendrocygna viduata* from Nelspruit in South Africa. Unfortunately the study had a very small sample size of two ducks, the transmitters functioned for less than eight months and they did not evaluate daily movement patterns. More recently, Cappelle *et al*. (2011) used satellite telemetry to study the movement patterns of Garganey *Anas quequedula*, Comb Duck *Sarkidiornis melanotos*, White-faced Duck and Fulvous Duck *Dendrocygna bicolor* in parts of central and West Africa in order to understand influenza transmission pathways, but they too did not evaluate daily movement patterns.

As a first contribution to a more detailed understanding of daily movement patterns for an Afrotropical duck, I analysed data for Egyptian Geese during the flightless moult and flying periods in two different rainfall regions (summer and winter) of South Africa.

Methods and Materials

Field methods and sites

Egyptian Geese that had just completed flight-feather moult were caught and tagged with solar-powered, satellite GPS transmitters (Platform Transmitter Terminals, PTTs) at Barberspan Nature Reserve (26º 33´ S, 25º 37´ E) in the North West Province and at Strandfontein Wastewater Treatment Works (34º 05´ S, 18º 32´ E) in the Western Cape Province, South Africa, between 15 January and 4 December 2008 (Table 1). Birds were caught using maize-baited, walk-in traps placed near the water's edge.

Barberspan is situated in the north east of South Africa in a transitional area between the grassland and savanna biomes. Barberspan Nature Reserve itself is surrounded by crop and sunflower farms (Mucina et al. 2006). The area is semi-arid, receives summer rainfall and consists of undulating plains rising to 900 m above sea level. The area is characterized by large depressions, or pans, where rainfall collects during the wet summer season.

Strandfontein Wastewater Treatment Works is situated along the Atlantic Ocean coast in the south-western corner of the country. The area has a Mediterranean climate and vegetation, with wet winters and warm, dry summers, and shrubs dominate the plant communities. The surrounding area has strong gradients of human population density, ranging from a large city (Cape Town) to sparsely settled agricultural and rural areas. The dominant forms of agriculture in the area are viticulture and crop cultivation.

Twenty-one Egyptian Geese (mean weight 2299 g), eight at Barberspan and 13 at Strandfontein, were tagged with 30 g solar-powered satellite GPS transmitters (hereafter referred to as 'transmitters') manufactured by Microwave Telemetry (http://www.microwavetelemetry.com). Blood samples were taken from each tagged bird to determine their sex (Griffiths *et al*. 1998).

Transmitters were attached using backpack harnesses, based partly on the design of Smith and Gilbert (1981), and comprising a front neck loop and a back body loop of Teflon ribbon straps. The front straps were placed on the shoulders of the bird and the back loop straps were passed under the flank feathers and around to the breast. More details of the attachment method are described in Cumming and Ndlovu (2011) [See Appendix 1].

Data analysis

Transmitters were set to capture data every two hours (i.e., 12 points per day). The transmitters provided high-quality GPS fixes accurate to within 20 m. Distances covered between successive fixes were computed as straight-lines. Satellite reception in southern Africa is excellent and detailed visual inspection of the data indicated no highly erroneous locations within the data set. In addition, the analysis used thousands of data points and the most important movements were relatively far, meaning that small errors in location would be both difficult to screen out and irrelevant to the final conclusions. Aside from removing testing locations and stationary points from the end of data series (i.e., where transmitters had fallen off or the animal was dead), I therefore did not undertake any data error screening or pre-processing.

I used general additive mixed models (GAMMs) to investigate distances moved by Egyptian Geese at different times of the day, considering gender, site, season and individual as potential influences on movement patterns. I compared distances moved by geese in the winter-rainfall, south-west region of South Africa with movements in a semi-arid, summer rainfall region in northern South Africa. In the winter-rainfall region, water bodies are less patchy in space and more predictable over time than in the summer-rainfall region (Bennetts and Kitchens 2000). General linear models (GLM) were inappropriate for these analyses because distance moved was non-linearly related to time of day (Figure 1) and two of the covariates, sex and site, were binary variables. GAMMs overcome the limitations of GLMs by identifying nonlinearities using flexible, nonlinear spline smoothing, but preserve the ability of GLMs to construct complex models with easy interpretability of predictor-response relationships (Hastie and Tibshirani 1990; Wood 2006). GAMMs also have an advantage over GLMs in that the more complex stochastic structure allows treatment of autocorrelations and repeated measures situations in such a way as that avoid pseudo-replication and the subsequent effects of increasing type I error (Wood 2004).

One-way analysis of variance at 95% confidence interval level was also used to test for differences in daily distances moved between Barberspan and Strandfontein Egyptian Geese.

All analyses were carried out with the R system for statistical computing (R development Core Team 2010) using the vegan package ‘mgcv’ (Wood 2006).

Results

Eighteen of the 21 transmitters deployed functioned for more than 50 days or gave over 100 fixes. In my analysis, I considered data from only these 18 birds and left out the other three transmitters (PTTs 77120, 77117 and 77122.1). Seven transmitters (PTTs 77118.2, 77127, 77128, 77130, 77132, 77134 and 77135) were still functional and transmitting on the 31st of October 2010, the day I closed the data set (Table 1).

Table 1. Summary of satellite-tagged Egyptian Geese. Column headings are as follows: PTT = transmitter identity; Site = location at which transmitter was attached; Distance = maximum distance, in kilometres, moved from tagging site (i.e. linear distance between tagging location and the farthest fix); Days = total period over which transmitter was active; Fixes = numbers of reliable locations obtained. Sex codes are: M, male; F, female. Site codes are: Bar, Barberspan; Str, Strandfontein.

PTT	Sex	Site	Start date	End date	Distance	Days	Fixes
77122.2	M	Bar	07 Jun 08	06 Mar 10	409	637	2 861
77127	M	Bar	07 Jun 08	31 Oct 10	702	976	6 401
77128	F	Bar	07 Jun 08	31 Oct 10	676	976	6 323
77129	M	Bar	07 Jun 08	19 May 10	639	346	3 491
77130	M	Bar	07 Jun 08	31 Oct 10	285	976	5 018
77131	M	Bar	07 Jun 08	13 Oct 09	6	493	1 209
77132	F	Bar	07 Jun 08	31 Oct 10	661	976	4 960
77094	F	Str	15 Jan 08	04 Sep 09	28	598	4 066
77095	F	Str	15 Jan 08	12 Dec 09	46	697	3 399
77116	F	Str	16 Jan 08	01 Apr 08	5	76	803
77118.1	M	Str	17 Jan 08	15 Jan 09	132	364	483
77118.2	F	Str	17 Jan 09	31 Oct 10	89	652	6012
77119	M	Str	17 Jan 08	16 Jun 08	65	151	805
77121	M	Str	18 Jan 08	15 Apr 08	1	88	284
77123.2	M	Str	04 Dec 08	29 Nov 09	41	360	1 894
77133	M	Str	04 Dec 08	24 Nov 09	103	355	2 534
77134	F	Str	04 Dec 08	31 Oct 10	107	696	5 400
77135	M	Str	04 Dec 08	31 Oct 10	104	696	6 755

Movement during moult (moulters)

Moulting Egyptian Geese all remained at the wetland where they were captured. The GAMM analysis showed that (correcting for random factors), the distance moved by moulting birds was significantly associated with time of day (F = 44.45, $p < 0.001$, R-sq.adj = 0.2898, n = 5798); site had a significant effect (F = 0.04, p = 0.034); and sex had no effect (F = 0.036, p = 0.849). Daily distances moved by individual moulting birds at each moult site did not differ significantly (Barberspan: F = 26.35, p = 0.052, n = 5; Strandfontein: F = 44.21, p = 0.06, n = 11) and Barberspan birds moved farther on a daily basis than did Strandfontein birds (4.32 ± 1.01 km and 1.95 ± 0.76 km respectively, F = 119.05, $p < 0.001$, n = 2). Across both sites, Egyptian Geese showed a bimodal pattern of movement activity, with movement peaking between 04h00 and 06h00 (when birds moved furthest) and a lesser peak between 16h00 and 18h00 (Fig. 1). Barberspan sunrise was between 06h00 – 07h00 and sunset was between 18h00 – 19h00 while Strandfontein sunrise was between 05h00 – 06h00 and sunset was between 19h00 – 20h00 during moult.

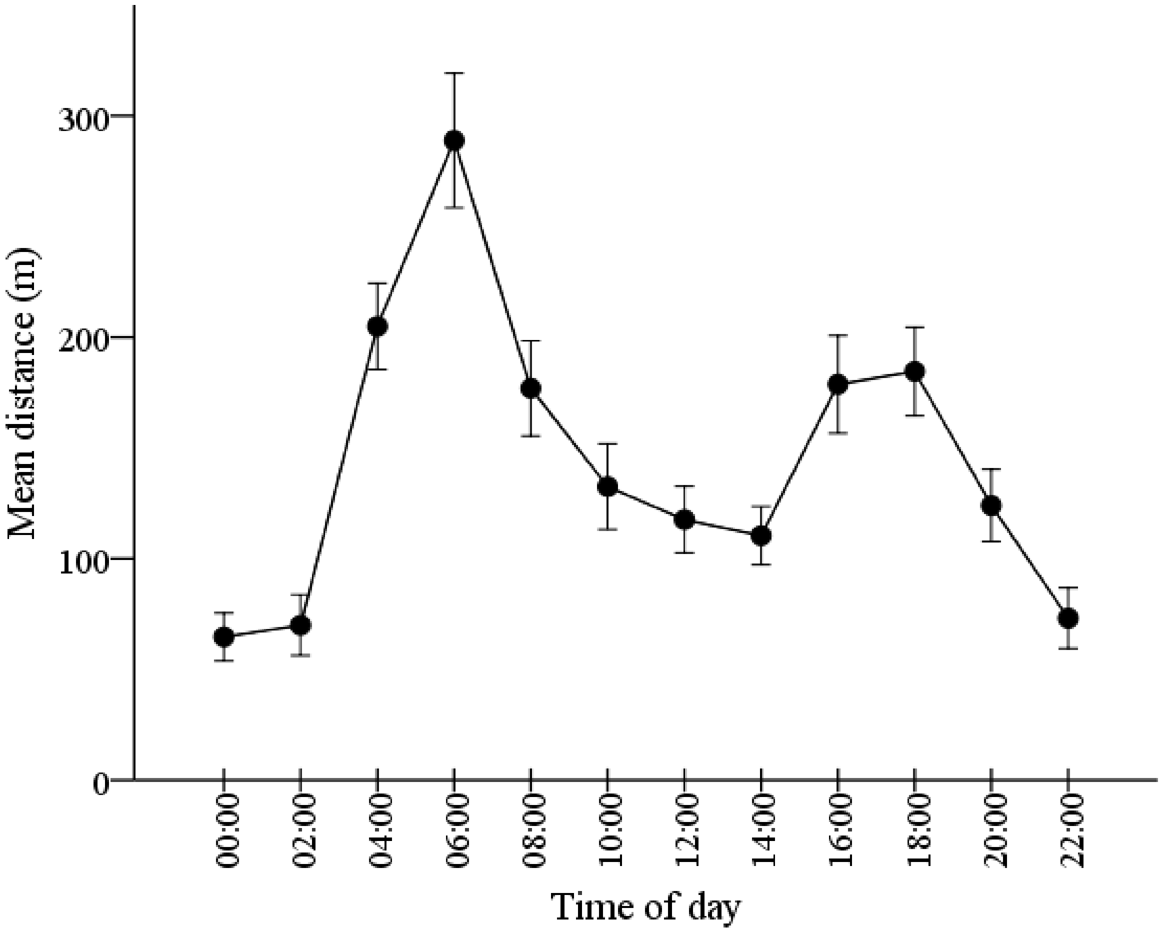

Figure 1. Mean distances moved by moulting Egyptian Geese [± Standard Error, 95% CI] as a function of time of day. Data are combined for Barberspan and Strandfontein.

Movement of non-moulting Egyptian Geese (outside the moult period)

One of the eight Barberspan birds, PTT 77177.1 was killed by a Black-backed Jackal *Canis mesomelas* at the site six days after release. Of the six Barberspan transmitters that functioned for at least 339 days, four birds dispersed maximum distances of over 600 km and the two others moved maximum distances of 409 km and 285 km away from Barberspan (Table 1). By contrast, none of the 11 Strandfontein-tagged birds travelled more than 132 km from the moult site. Four birds dispersed maximum distances of 103 – 132 km, while the remainder travelled less than 90 km from Strandfontein. PTT 77120 malfunctioned 27 days after deployment, by which time the bird had not moved away from Strandfontein, but the transmitter was recovered more than a year later when the bird was shot by a farmer at Stellenbosch, 65 km from Strandfontein.

The GAMM analysis showed that time of day (F = 18.85, $p < 0.001$), bird location (entered in GAMM as a random variable; Latitude * Longitude) (F = 14.47, $p < 0.001$) and month after moult (F = 7.81, p = 0.002) were the best predictors of distance moved (R-sq.adj = 0.129, n = 24271): sex of the bird had no effect (F = 36.99, p = 0.52). Daily distances moved by individual birds tagged at the same moulting site differed significantly (Barberspan F = 4.25, $p < 0.001$, n = 8; Strandfontein: F = 54.16, $p < 0.001$, n = 9). Egyptian Geese that moulted at Barberspan moved significantly further (14 ± 0.71 km) than those from Strandfontein (6.6 ± 0.19 km, F = 83.85, $p < 0.001$).

On a daily basis, most non-moulting Egyptian Geese moved between one and 10 km (Table 2). There were 80 instances when birds tagged at Barberspan moved more than 100 km in a day. Of these, 21 occurred in the first month after moult, 16 in the 11th month after moult and the other 43 occurred in the period between the 2nd and 10th month after moult. By contrast, on only two occasions did a Strandfontein-tagged bird move more than 100 km in a day, and both movements were by the same bird: PTT 77118.1 (just before and soon after moult).

Table 2. Summary of daily distances moved by non-moulting Egyptian Geese.

Tagging site	Number of daily distances recorded	Longest daily distance	Percentage distribution of daily distances (%)			
			0–1 km	1–10 km	10–100 km	>100 km
Barberspan	2745	664 km	15.95	57.49	23.45	3.11
Strandfontein	2245	225 km	14.16	65.26	20.49	0.09

Egyptian Geese that were tagged at Barberspan moved farthest in the morning, between 04h00 and 08h00 (Fig. 2). Egyptian Geese tagged at Strandfontein moved farthest in the early morning (04h00–08h00) and late afternoon (16h00–18h00; Fig. 3). Least movement occurred at night between 20h00 and 02h00 and there was also a lull in activity during the middle of the day (10h00–16h00).

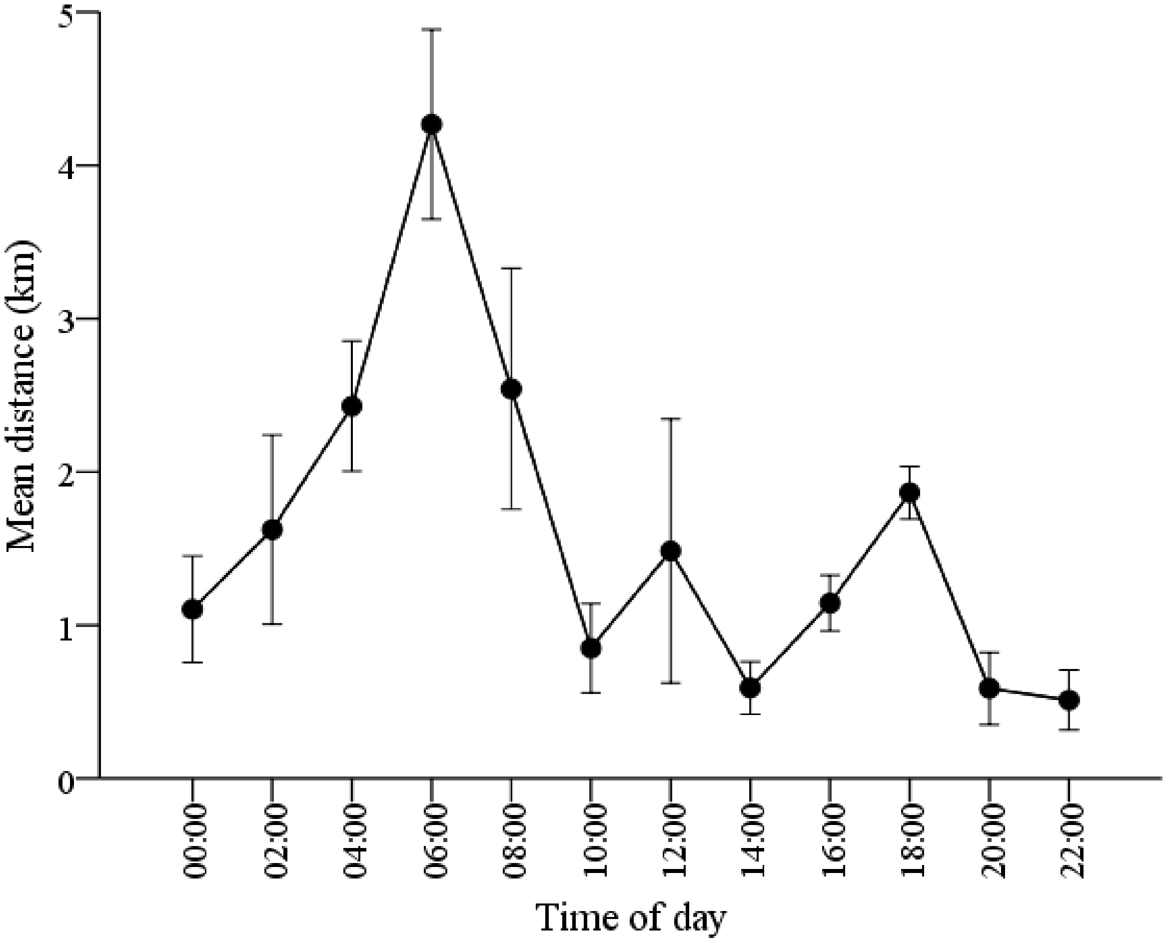

Figure 2. Mean distances moved by non-moulting Egyptian Geese that had moulted at Barberspan [± S.E., 95%] as a function of time of day.

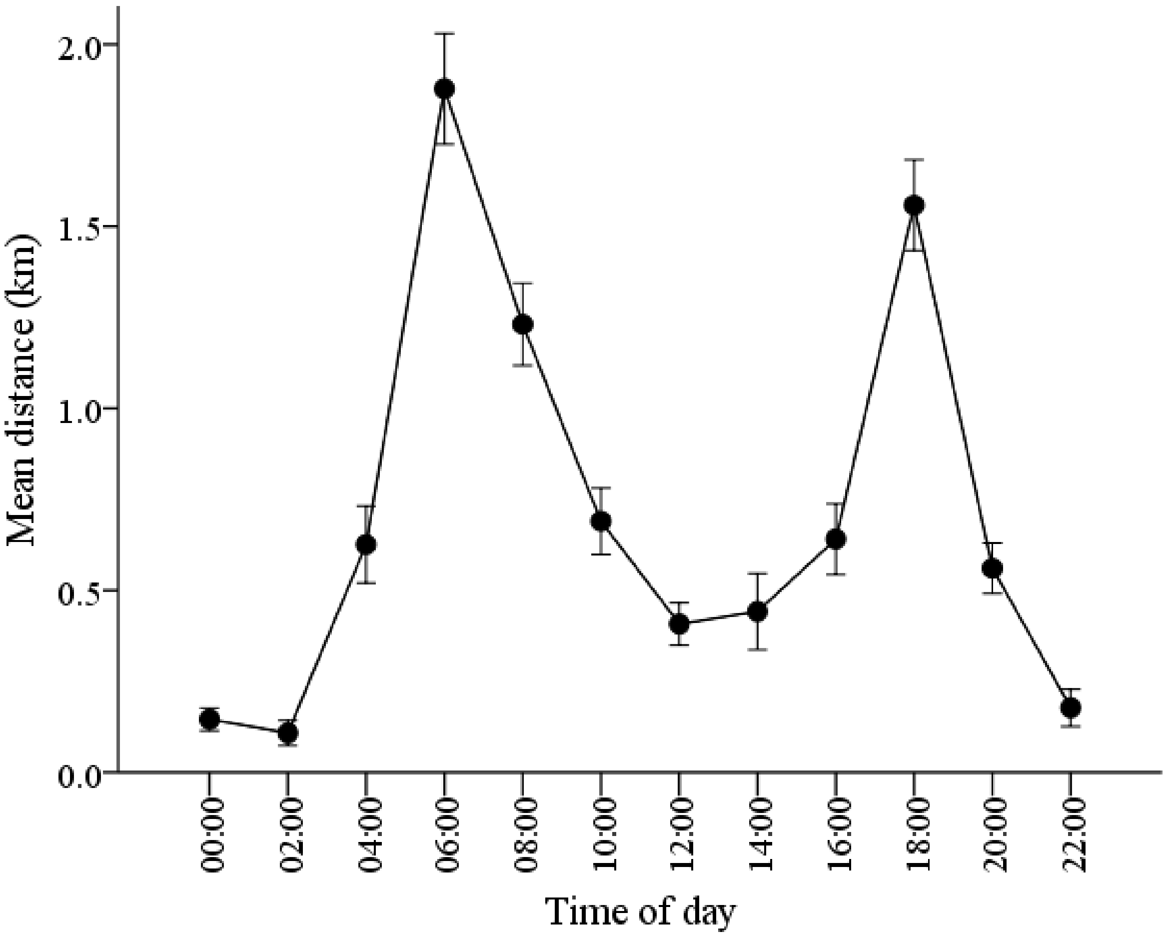

Figure 3. Mean distances moved by non-moulting Egyptian Geese that had moulted at Strandfontein [± S.E., 95% C.I] as a function of time of day.

In addition to being linked to time of day, movement patterns were linked to the timing of moult. Birds from Barberspan travelled the farthest per day immediately before moult (month 11), with a much less marked peak in daily travel distance immediately after moult (month 1; Fig. 4). For the remainder of the year, daily movement distances showed little variability (Fig. 4).

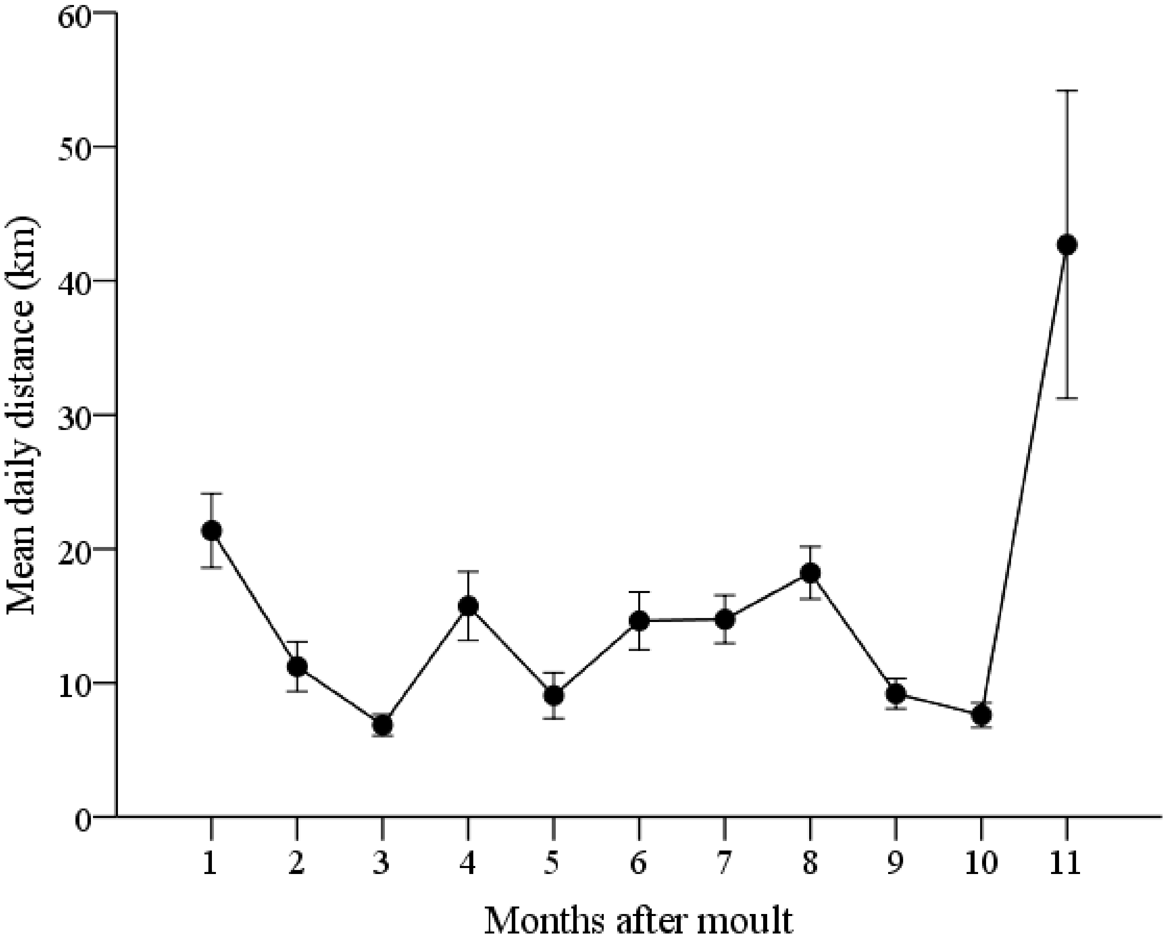

Figure 4. Mean daily distances moved by Egyptian Geese that moulted at Barberspan [± 95% C.I] in relation to the timing of moult.

Strandfontein-tagged birds moved farthest in the month immediately following moult, but these distances were not significantly different from the subsequent four months. In the sixth months after moult, daily movement distances travelled decreased sharply, then steadily increased until immediately prior to moult (Fig. 5).

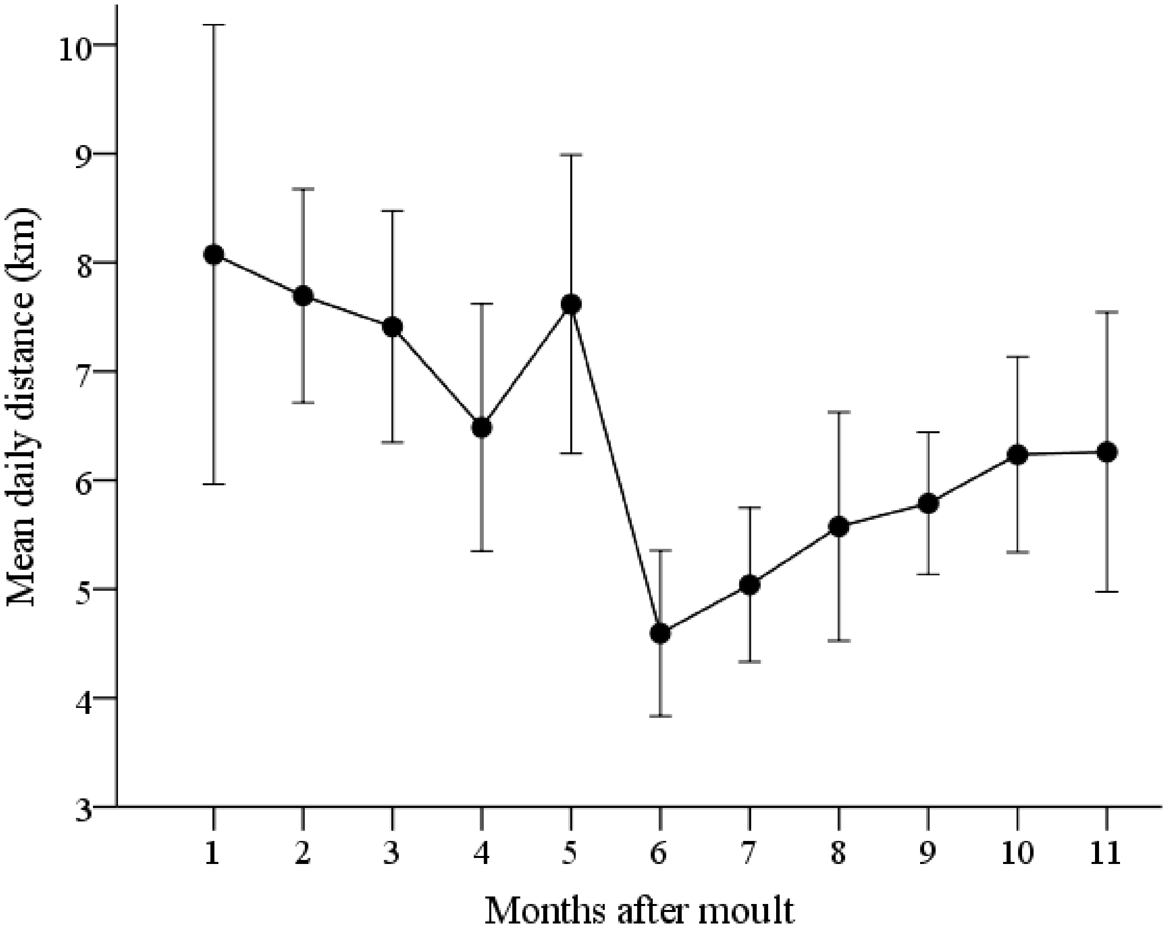

Figure 5. Mean daily distances moved outside the moult period, by Egyptian Geese that had moulted at Strandfontein [± Standard Error, 95% CI] in relation to the timing of moult.

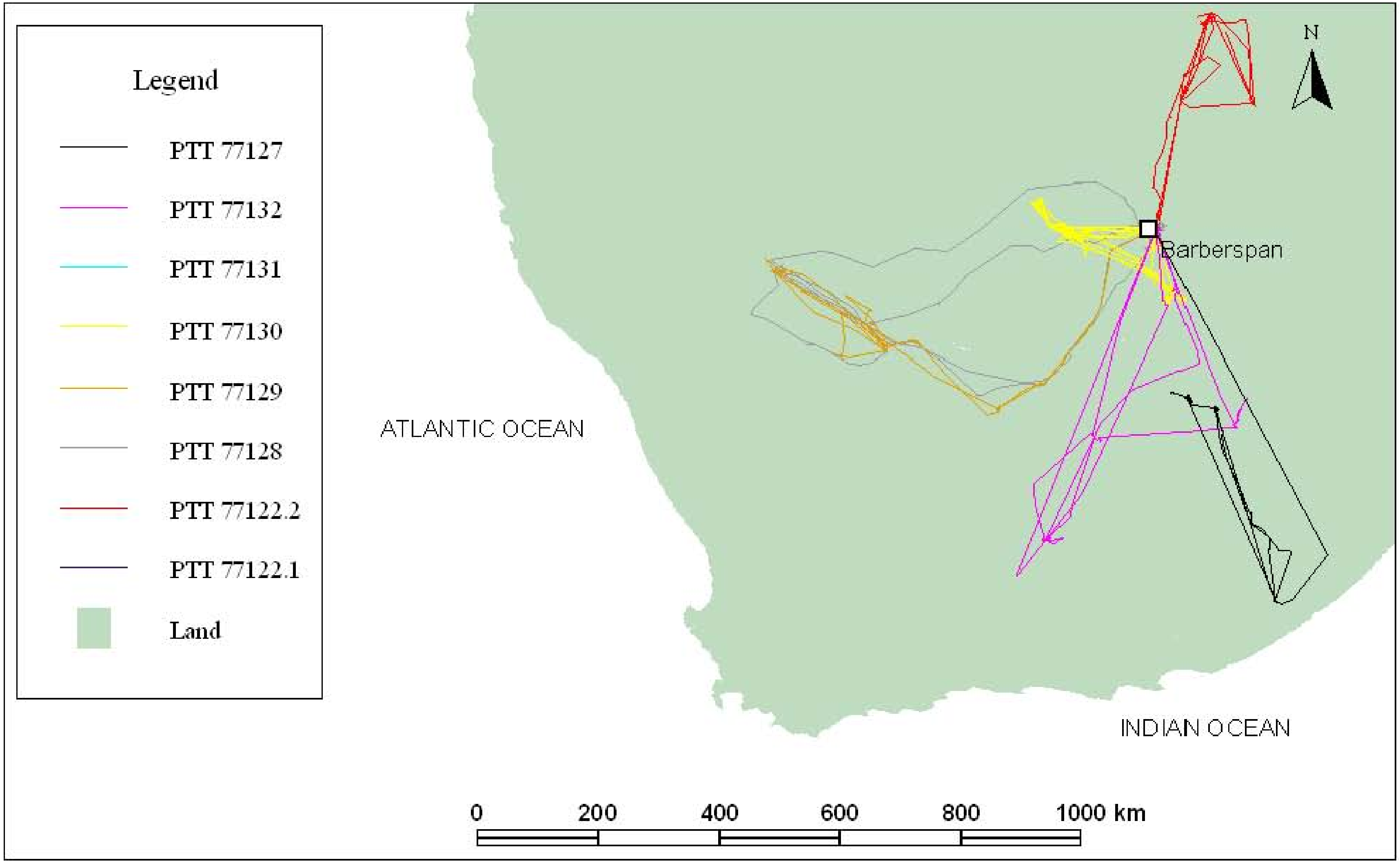

Figure 6. Movement paths of eight Egyptian Geese tagged and released just after moult at Barberspan Nature Reserve, South Africa.

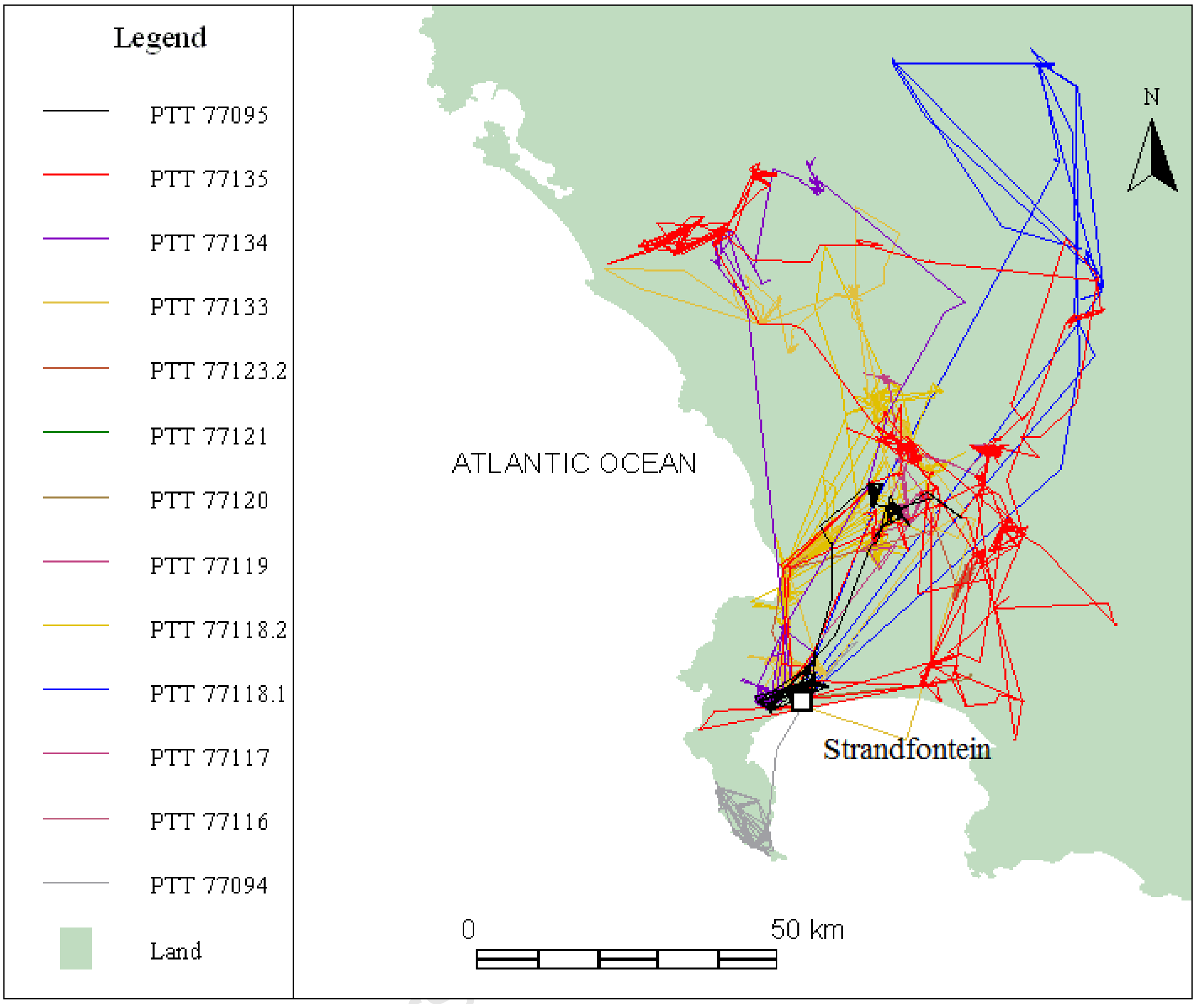

Figure 7. Movement paths of 11 Egyptian Geese tagged and released just after moult at Strandfontein Wastewater Treatment Works, South Africa.

Birds from Barberspan never returned to their moulting site outside the moulting season, whereas non-moulting, satellite-tagged birds from Strandfontein returned to the moulting site an average of 2.31 times per year. Each visit to Strandfontein lasted between two and eight hours. Three birds (PTT 77128, 77129 and 77122.2) that moulted at Barberspan showed breeding site and moult site fidelity, returning to the same post-moult (and breeding) area each year. Each bird bred at a different locality (Fig. 6).

Discussion

Moulting Egyptian Geese at Barberspan travelled farther on a daily basis than moulting birds at Strandfontein. This is a direct reflection of the differing sizes of the two wetlands. Barberspan has a water area of 1700 ha, compared with only 319 ha at Strandfontein. Flightless Egyptian Geese at Barberspan therefore swim longer distances from the island or from the centre of the pan where they spend the night, to graze ashore. Moulters at both sites were most active at dawn, with a second, smaller peak in activity around dusk (Fig. 1). During the midday period of inactivity, birds spent most time close to the shore, loafing and preening (as observed during the colour-ring searches; Chapter 5). These crepuscular patterns of activity and nocturnal retreats to safety are compatible with the behaviours being driven by the risks posed by nocturnal predators such as Black-backed Jackals. As the predators return to their dens at dawn (Loveridge and Macdonald 2003), the birds come ashore to graze.

At the end of moult, Egyptian Geese from both sites dispersed away from their moulting sites. Most of the longest daily distances travelled by individuals happened within the first 10 days after completing moult and just before the moult cycle was due to restart. The long-distance travel paths (Figs 6 and 7) summarise these movements and provide compelling evidence for these birds undertaking moult migrations. The existence of moult migrations by waterfowl in southern Africa has long being suspected (e.g. Geldenhuys 1981; Milstein 1993) but has never been proven, not least because of the disparate directions in which the migrating birds move (Figs 6, 7). This study thus provides empirical evidence of such migrations and some indication of the distances that birds travel during these migrations (see also Cumming *et al*. 2012). It is not clear why Egyptian Geese tagged at Barberspan travel such long distances (over 600 km in the case of four individuals) to moult again at Barberspan, given that these birds bypass several seemingly suitable moulting sites *en route* (although it is the bypassing of apparently suitable resources that some authors consider a necessary precondition for the definition of true migration - Dingle 1996). It is very likely that similar migrations take place among South African Shelducks *Tadorna cana*, a species whose global population moults at only a few wetlands (Geldenhuys 1981).

While general seasonal patterns of movement in relation to the timing of moult were concordant between birds from the summer- and winter-rainfall regions, there were considerable differences in patterns of dispersion between Barberspan and Strandfontein birds (Table 1; Figs 7, 8; Cumming *et al*. 2012).

The daily distances travelled can be divided into short (≤ 10 km) and long distances (> 10 km). Short flights reflect foraging within a discrete area, typically between a roosting wetland and a grazing pasture, or between wetlands. Long flights were predominantly undertaken at night (88%), and these were associated with semi-permanent changes in location. The pattern of daily movement is strongly skewed, with over 70% of flights being over distances of less than 10 km and longer flights over 10 km, being uncommon (Table 2).

Birds tagged at Strandfontein typically flew back to Strandfontein at least twice between moults, but would spend on average four hours there before returning to their previous location. Birds at Barberspan, however, did not return until the next moulting season.

Simmons *et al*. (1998) and Roshier *et al*. (2006) suggested that waterfowl could find temporary pans using visual cues such as thunder-cloud formations that precede massive rain fronts. Whilst this may be true over spatial scales of less than *ca* 100 km, it seems an unlikely explanation for night-time flights by Egyptian Geese that took them to isolated water bodies in dry regions of Botswana, Namibia and the Northern Cape Province. Rather, these observations suggest that Egyptian Geese at Barberspan interact with their environment at very broad scales and that their long-distance flights are based on experience and spatial memory (Nathan *et al*. 2008; Cumming *et al*. 2012). Birds from Strandfontein, by contrast, travelled shorter distances and more frequently, thus more constantly ‘sampled’ their surroundings (Nathan *et al*. 2008; Cumming *et al*. 2012).

There were no clear, sex-related differences in any of the movement parameters examined in this study. Given that Egyptian Geese form long-term pair bonds (Milstein 1993), this is not unexpected. Birds from the resource-dense, winter-rainfall area remained within the vicinity of their moulting site, while birds from the semi-arid, resource-sparse summer-rainfall area travelled much further afield and did not return to their moulting site between moults. Once Egyptian Geese from Barberspan had arrived at their post-moult destinations, most of their daily movements were over short distances, similar to the movement patterns of White-faced Ducks in the same region (Petrie and Rogers 1997). White-faced Ducks stay several months in one area and frequently undertake short daily flights between wetlands or from wetlands to terrestrial foraging sites (Petrie and Rogers 1997). A similar pattern of short-distance daily movement is also shown by Grey Teal in Australia. These birds travel mean distances of between 2.9–25.2 km per day (Roshier *et al.* 2006), similar to the distances travelled daily by non-moulting Egyptian Geese in the present study. The longest daily distance recorded for a Grey Teal is 332 km (Roshier *et al*. 2006), exactly half the longest daily distance travelled by an Egyptian Goose (Table 2). A Comb Duck tagged in Malawi had a displacement distance of 655 km in 285 days, similar to displacement patterns of Egyptian Geese from Barberspan (Cappelle *et al*. 2011, Appendix 1).

Barberspan birds undertake long-distance movements that cannot be interpreted as ‘random wanderings’ between adjacent wetlands (Fig. 7). Movement of Strandfontein birds, on the other hand, do appear to be random and over short distances (Fig. 8), fitting the expectations of nomadism (Bennetts and Kitchens 2000). It appears that the proximate cues for movement by Strandfontein birds function at the local landscape level, whilst birds from Barberspan rely mainly on memory (possibly genetically ‘hard-wired’) when travelling long distances. A similar dichotomy in movement patterns has been documented among dispersing, juvenile African Black Oystercatchers *Haematopus moquini*. Some move by a process of diffusion dispersal, covering fairly short distances, while others both bypass apparently suitable resources and traverse extensive stretches of inhospitable coastline to aggregate at sites >1500 km from their birthplaces and outside the species' breeding range (Hockey *et al*. 2003). In the case of the oystercatchers, it has been mooted that short-distance movements may be driven by proximate environmental cues whereas longer-distance movements are more likely to be under genetic control (Hockey *et al*. 2003). My results suggest that Egyptian Geese have equally divergent movement strategies that may be determined more by where they moult than where they breed. The fact that they are over-dispersed when breeding and under-dispersed when moulting suggests that suitable moulting sites, certainly for those birds that live in the semi-arid regions, may be the limiting resource that ultimately determines their life history.

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CHAPTER 7

Synthesis

Introduction

The annual life-history events of waterfowl in north-temperate regions are driven primarily by seasonal variations in temperature, which are predictable in their timing and intensity (Oring and Sayler 1992). In contrast, the spatio-temporal availability of adequate resources to waterfowl in the semi-arid regions of southern Africa is determined by rainfall (wet and dry rhythms), which is extremely variable in timing and intensity (Oring and Sayler 1992; Petrie 1998). The inherent variability and unpredictability of the southern African environment is expected to influence waterfowl life-history strategies (Maclean 1976; Petrie and Rogers 2004), which themselves may differ from those of their north temperate counterparts.

The aim of this study was to understand the environmental influences on flight-feather moult and movement strategies of southern African waterfowl. However, attempting to study all southern African waterfowl is a daunting task because of the number of species and the diversity of habitats they occupy. Furthermore, intra- and interspecific variation in response to latitude gradients and fluctuations in environmental conditions within waterfowl (Cumming *et al*. 2012) makes detailed predictions of strategies at the species level even more complex. To overcome this challenge I selected six African duck species that are fairly common and occur in most parts of South Africa, namely; Egyptian Goose *Alopochen aegyptiaca*, Spur-winged Goose *Plectropterus gambensis*, South African Shelduck *Tadorna cana*, Yellow-billed Duck *Anas undulata*, Red-billed Teal *A. erythrorhyncha* and Southern Pochard *Netta erythrophthalma*. I also studied the Egyptian Goose in more detail as a model species to investigate the underlying responses of Afrotropical waterfowl to stochastic environmental conditions and explore the life-history strategies they have evolved to cope with this variability. My research therefore combined behaviour, ecology, moult biology and movement into a broad-scale assessment of the responses of Egyptian Geese (as a model species for other southern African waterfowl) to environmental variation in South Africa.

Another key motivation for this study was that it provides an opportunity to compare and contrast the life-history strategies of species living in a semi-arid region of the southern hemisphere with those of waterfowl living in north temperate environments. Studies of waterfowl biology have been biased towards the northern hemisphere, where the availability of aquatic habitats and forage are relatively predictable throughout the annual cycle. This research has produced several ostensible paradigms explaining duck moult and movement patterns. I was interested in the extent to which these paradigms were applicable in a southern African context, given the marked environmental differences between the two regions.

I chose two study sites to represent the extremes of seasonal rainfall conditions experienced by southern African waterfowl in South Africa. Barberspan is situated in a subtropical, summer-rainfall region and Strandfontein is located at a temperate, winter-rainfall region.

Key findings

To optimise survival during flight-feather moult in stochastic, semi-arid conditions, southern African waterfowl have evolved physiological strategies that are different from those of north-temperate waterfowl. I investigated whether, or to what degree, body condition, flight-feather growth, pectoral muscle mass and organ (gizzard, liver and heart) mass of Egyptian Geese in southern Africa showed phenotypic flexibility over the short period of flightless moult. This component of the study revealed that body mass, body condition, and pectoral muscle, gizzard and liver size varied significantly during moult (Chapter 2). Body mass and condition started to decrease soon after flight feathers were dropped and continued to do so until the new feathers were at least two-thirds grown, after which birds started to regain body mass and condition. Pectoral muscle mass decreased once moult started and continued to do so until the flight feathers were at least one-third grown, after which pectoral muscle mass started to increase. The regeneration of pectoral muscles during moult thus commenced before birds started to gain overall body mass. Gizzard mass started to increase soon after the onset of moult, reaching a maximum when the flight feathers were two-thirds grown, after which gizzard mass again decreased. Liver mass increased significantly as moult progressed, but heart mass remained constant throughout. Flight-feather growth was initially rapid, but slowed towards the completion of moult. This substantial phenotypic flexibility appears to have evolved to allow moult to be completed as rapidly as possible. This is presumably advantageous in terms of both predator avoidance and minimising the period of nutritional stress.

I then built on detailed findings of Egyptian Geese phenotypic flexibility and tested for the existence or extent of interspecific variation in the body masses and pectoral muscle sizes of other southern African waterfowl during flight-feather moult (Chapter 3). Spur-winged Geese and South African Shelducks underwent atrophy and subsequent regeneration of the pectoral muscles, with body mass decreasing at the onset of moult and subsequently stabilising from the time when flight feathers were two-thirds grown. By contrast, the body masses of Yellow-billed Ducks and Red-billed Teals decreased from the onset of moult until the mid-point, but thereafter increased rapidly, returning to pre-moult levels by the time moult was completed. Southern Pochards (the only diving species studied) gradually lost mass from the start of moult almost until moult completion, at which time mass increased slightly. The interspecific differences in the moult strategies of southern African waterfowl as reflected in changes in body and pectoral muscle conditions are complex and presumably a result of differences in body size and foraging mode. However, it seems fairly certain that phenotypic flexibility in moulting Spur-winged Geese, South African Shelducks, Yellow-billed Ducks and Red-billed Teals is an adaptation that permits flight-feather moult to be completed as rapidly as possible.

Most southern African waterfowl moult in the middle of the dry season (with the exception of South African Shelducks in the summer-rainfall region - Chapter 4), when: (1) food abundance is low; (2) water bodies that provide sufficient refuge for a flightless duck from predators are scarce; and (3) the likelihood of a moulting pan drying up during low-rainfall years is potentially high. The need for such a strategy is likely precipitated by a combination of (a) environmental aridity and stochasticity; (b) predation risk, and (c) the need to relocate to high-quality feeding areas as soon as possible. However, southern Pochards displayed a unique, steady, body-mass loss during moult that cannot be explained as an adaptation to reduce wing-loading or a strategy to ensure a rapid completion of moult: rather, it suggests that moult is energetically costly for this species.

I also studied the interactions of environmental conditions and the timing of breeding and moult in southern African waterfowl (Chapter 4). I tested the hypothesis that moult is the most energy-costly stage of the annual cycle and all other life-history stages are built around the timing of moult. I investigated how the timing of rainfall relates to the timing of breeding and moult in Egyptian Goose, Spur-winged Goose, South African Shelduck, Yellow-billed Duck and Red-billed Teal at two sites in South Africa with seasonally differing rainfall regimes (one summer, one winter). I further compared and contrasted the timing of breeding and moult in waterfowl in southern Africa, south-eastern Australia, Europe and North America. In general, southern African waterfowl bred in the wet season and moulted during the dry season. South African Shelduck in the summer rainfall area were an exception, breeding in the dry season and moulting during the wet season. There was a substantial lag period between peak breeding and peak moult in southern hemisphere species, the longest lag being that by birds in the summer-rainfall area. In comparison, northern hemisphere ducks breed and moult during the warm season (Kear 2005), and the lag period between peak breeding and peak moult is short (Chapter 4: Table 3), to the point where breeding and moulting overlap in northern hemisphere geese (Ely 2003; Kear 2005).

Waterfowl in southern Africa, south-eastern Australia, Europe and North America thus appear to time their breeding to coincide with peaks in the availability of both food and breeding sites. Southern African species, with the exception of the hole-nesting shelduck, breed during the wet season, regardless of whether this falls in summer or winter (Chapter 4). Northern hemisphere species breed at the warmest time of year, when days are long and food production is high (Svensson 1995). Northern hemisphere species also start and complete moult before food and the availability of aquatic habitats approach their winter minima. Thus, both breeding and moult are, in essence, compressed to within a single season. By contrast, southern hemisphere waterfowl delay the onset of moult until the height of the dry season, when food and aquatic habitats are scarce. I hypothesise that this stems from a need to minimise the risk of the chosen moult site drying out before moult is completed. Moulting birds are thus minimising the risk of predation, but at the potential cost of being food-stressed. Southern African waterfowl compensate for the latter to some extent by exhibiting a remarkable level of phenotypic flexibility that is geared towards minimising the flightless period (Chapters 2, 3).

Like previous studies (Shewell 1959; Geldenhuys 1981; Milstein 1993; Petrie and Rogers 2004), my results also show that southern African waterfowl concentrated in large numbers at both of my study sites, mainly to moult (Chapters 4, 5, 6). The move to permanent wetlands in the dry season when ephemeral pans dry out coincides with the timing of moult in Spur-winged Geese, Egyptian Geese, Yellow-billed Ducks and Red-billed Teals (Chapter 4).

Therefore, it is reasonable to conclude that permanent wetlands offer the best sites for moult, whereas ephemeral pans satisfy breeding and foraging requirements during the wet season: large, permanent wetlands like Barberspan and Strandfontein are not the preferred foraging and breeding habitats for waterfowl (Maclean 1976). Smaller, ephemeral pans, as suggested by earlier studies, are more productive and important for foraging and breeding (Dean 1978; Geldenhuys 1981; Milstein 1993).

I used telemetry and colour rings to investigate moult-site fidelity and dispersal distances of Egyptian Geese from moult sites (Chapter 5). There were 12.0% and 29.7% colour-ring return rates for geese ringed at Barberspan and Strandfontein, respectively. Four of five moulting birds tagged at Barberspan with satellite transmitters returned to Barberspan to moult the following year. Two of these same birds moulted at Barberspan in the third year while the other two birds moulted elsewhere. Only one of seven Egyptian Geese satellite-tagged at Strandfontein returned to the same site to moult the following year. One bird moulted at a nearby pan (5 km away), two moulted at estuaries less than 30 km from Strandfontein and one bird moulted at a farm dam 56 km away. Telemetry and a ring-resighting model indicated moderately high moult-site fidelity in Egyptian Geese, despite the anthropogenic modification of South Africa’s current landscape, particularly the increased number of permanent, artificial water bodies, which is expected to lower moult-site fidelity. While lack of environmental predictability has dictated that waterfowl living in semi-arid regions moult at traditional, permanent wetlands, an increasing number of impoundments, especially in the arid west of South Africa, has allowed some birds to be less faithful to traditional moulting sites and to explore alternatives (Chapters 5, 6). Moult site fidelity (as indicated by tracking data rather than colour-ring return data) is greatest in landscapes where permanent wetlands are least available. Despite the fact that the landscape, especially in the west of South Africa, has been altered and now contains more potential moult sites than historically, some traits that are evolutionarily adaptive to a stochastic environment have persisted, such as (1) moderate to high moult-site fidelity despite the availability of alternatives; and (2) phenotypic changes during flight-feather moult geared to shorten the flightless moult period.

In the final chapter I document the use of satellite telemetry to record the daily movements of Egyptian Geese (Chapter 6). Moulting Egyptian Geese all remained at the same wetland during moult and their daily movement patterns were similar at both study sites, with crepuscular activity patterns and nocturnal retreats to safety. At the end of moult, Egyptian Geese from both sites rapidly dispersed away from their moulting sites. There were considerable differences in patterns of dispersal between birds from the summer-rainfall site and those from the winter-rainfall site. Barberspan birds undertook long-distance (mean 482.57 ± 99.21 km) movements that cannot be interpreted as 'random wanderings' between adjacent wetlands. Most of the longest daily distances travelled by Barberspan birds happened within the first 10 days after completing moult and just before the moult cycle was due to restart, implying that these were periods of directed travel rather than local nomadism. These pre- and post-moult movements are best described as moult migrations. By contrast, pre- and post-moult movements of Strandfontein birds appeared to be random and over short distances (mean 52.28 ± 17.83 km), best explained as nomadism. It therefore appears that the proximate cues for movement by Strandfontein birds function at the local landscape level, whilst birds from Barberspan rely mainly on memory (and are possibly genetically 'hard-wired') when undertaking moult migrations (Chapter 6). The differences in distances moved to preferred moulting sites between populations within different rainfall regions reflect the differences in the dispersion of permanent wetlands between the south west and north east of South Africa, suggesting that landscape-scale features have some influence in shaping the moult migration patterns of Egyptian Geese. While the findings confirm some aspects of known waterfowl ecology, it is important to note that (1) not all Egyptian Geese moult every year; (2) some Egyptian Geese exhibit high moult-site fidelity whereas others within the same local population do not; and (3) that Egyptian Geese from the two sites show different movement patterns, despite the ringing evidence that there is some exchange of birds between these two sites (Underhill *et al*. 1999).

Conclusions

It is reasonable to conclude that (1) substantial phenotypic changes during moult; (2) wet-season breeding and dry-season moult (except for shelducks in the summer-rainfall region); and (3) moult-site fidelity to permanent pans, are significant evolutionary adaptations that enhance the survival of waterfowl in semi-arid environments.

There is still much to learn about the moult biology and movement patterns of other waterfowl species living in the semi-arid regions of southern Africa. The few other studies that have focused on southern hemisphere waterfowl do, however, point to unique aspects of their biology and movement patterns that differ from those of migratory, northern hemisphere waterfowl (e.g. Petrie and Rogers 2004; Roshier *et al*. 2008; Cumming *et al*. 2012). My study demonstrates that semi-arid and north-temperate waterfowl have divergent moult and movement strategies. These differences seemingly stem from differences in environmental predictability (especially rainfall) that themselves ultimately determine the dispersion and persistence of suitable habitats and food (Petrie and Rogers 2004). Therefore, one of the key scientific contributions of this study is the recognition that many dominant biological paradigms derived from studies of north-temperate environments are not directly applicable to southern African waterfowl, e.g. northern hemisphere species moult when food and aquatic habitats are abundant whereas most southern hemisphere waterfowl moult during the height of the dry season, when food and aquatic habitats are few. Consequently, moulting Egyptian

Geese in South Africa undergo more substantial phenotypic changes during moult than those reported for ducks in the northern hemisphere. It is reasonable to expect that, as environmental uncertainty increases, the number of possible strategies to cope with this uncertainty will also increase. It appears that some of this potential variability is shown by southern African waterfowl. Intra- and inter-specific differences in the way species respond to the same regional environmental cues do exist (e.g. Cumming *et al*. 2012; Chapters 3, 4, 5, 6), manifested, for example, in subtle differences in phenotypic plasticity while moulting. Comparing the findings of this study and those of Petrie (1998) and Milstein (1993), I propose that the moult strategies and movement patterns of southern African waterfowl are shaped by a combination of individual responses to short-term (rainfall patterns that determine food availability and water levels) and longer-term (proliferation of man-made water bodies) availability of water in the landscape. In support of this, where anthropogenic changes to the aquatic landscape are the greatest (in the west of South Africa), waterfowl life histories (as exemplified by Egyptian Geese) appear to have deviated the most from what is presumed to have been the historical condition, especially with respect to movement patterns.

Directions for future research

A key result question that was not addressed in this study is whether there were age-specific differences in (1) phenotypic fluctuations during moult; (2) dispersal distances and movement patterns; and (3) the degree to which movement patterns are learned by sub-adults from older individuals, and whether they are genetically hard-wired or are influenced by the landscape. The next step in understanding these three unknown issues is (1) to evaluate age-linked differences in moult biology and movement patterns, and (2) to translocate adult and sub-adult birds from Strandfontein to Barberspan, and vice versa, to investigate if their movement patterns will change or whether they simply ’return home’.

I recommend that long-term, fine-scale research of the movements of other, less studied Afrotropical waterfowl such as South African Shelducks (which exhibit strong moult-site fidelity to only a few pans in South Africa), Spur-winged Geese (thought to be partially migratory - Hockey et al. 2005) and Comb Ducks *Sarkidiornis melanotos* (which have extensive movements between southern Africa and east Africa - Underhill et al. 1999) would prove valuable in identifying and conserving wetlands important to waterfowl life histories and for modelling zoonotic avian disease dynamics (e.g. avian influenza and West Nile virus).

Another important aspect for future research will be to establish the moult and movement biology of southern African waterfowl in areas receiving aseasonal rainfall (which cover a substantial amount of South Africa). This is the environment in which birds might be expected to show the greatest behavioural plasticity, but to date the extent of this plasticity is undocumented.

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APPENDIX

Published papers

Articles

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Phenotypic flexibility of a southern African duck *Alopochen aegyptiaca* during moult: do northern hemisphere paradigms apply?

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Phenotypic flexibility during moult has never been explored in austral nomadic ducks. We investigated whether the body condition, organ (pectoral muscle, gizzard, liver and heart) mass and flight-feather growth Egyptian geese *Alopochen aegyptiaca* in southern Africa show phenotypic flexibility over their 53-day period of flightless moult. Changes in body mass and condition were examined in Egyptian geese caught at Barberspan and Strandfontein in South Africa. Mean daily change in primary feather length was calculated for moulting geese and birds were dissected for pectoral muscle and internal organ assessment. Mean body mass and condition varied significantly during moult. Body mass and condition started to decrease soon after flight feathers were dropped and continued to do so until the new feathers were at least two-thirds grown, after which birds started to regain body mass and condition. Non-moulting geese had large pectoral muscles, accounting for at least 26% of total body mass. Once moult started, pectoral muscle mass decreased and continued to do so until the flight feathers were at least one-third grown, after which pectoral muscle mass started to increase. The regeneration of pectoral muscles during moult started before birds started to gain overall body mass. Gizzard mass started to increase soon after the onset of moult, reaching a maximum when the flight feathers were two-thirds grown, after which gizzard mass again decreased. Liver mass increased significantly as moult progressed, but heart mass remained constant throughout moult. Flight feather growth was initially rapid, but slowed towards the completion of moult. Our results show that Egyptian geese exhibit a significant level of phenotypic flexibility when they moult. We interpret the phenotypic changes that we observed as an adaptive strategy to minimize the duration of the flightless period. Moulting Egyptian geese in South Africa undergo more substantial phenotypic changes than those reported for ducks in the northern hemisphere.

Much of what we know about waterbirds comes from north-temperate latitudes where the availability of water is seasonally predictable and where water is seldom a limiting resource (Baldassarre and Bolen 2006). High predictability in resource availability has allowed north-temperate birds to fine-tune their life histories, dispersal strategies and physiological responses in a variety of ways to exploit this predictability to the fullest (Wingfield et al. 1992, Svensson 1995, Piersma and Drent. 2003, Bêty et al. 2004).

In areas where resource availability is less predictable, we may expect to find different responses by waterbirds to environmental fluctuations. In this context, southern African waterbirds offer an interesting case study. Much of southern Africa is arid and the region as a whole is considered "water scarce" (Harsch 1992). In addition to its aridity, the timing of rainfall events can be highly unpredictable (Tyson and Preston-Whyte 2000). Local variations in rainfall present a challenge for waterbirds, particularly ducks and geese (Anatidae) that need standing water of sufficient depth for foraging, predator avoidance and flight feather moult.

Ducks undergo a three- to five-week period of flightless moult, during which they replace all of their primary feathers. During this period, grazing ducks and geese generally have to remain close to water and occasionally venture onto land to feed on adjacent, low-quality forage (Halse and Skead 1983, Panek and Majewski 1990). Some of the physiological changes observed in north-temperate ducks and geese during moult have been associated with reductions in mass (Hanson 1962, Folk et al. 1966, Hohman et al. 1992, Fox and Kahlert 2005). Early studies attributed mass loss to nutritional stress caused by a scarcity of food (Hanson 1962, Folk et al. 1966). By contrast, Pehrsson (1987) proposed that mass reduction was an adaptive strategy to reduce wing loading and thereby shorten the flightless period. Recent studies of two north-temperate species, greylag goose *Anser anser* (Fox and Kahlert 2005) and common scoter *Melanitta nigra* (Fox et al. 2008) suggest that the reduction during moult in the mass of body organs associated with flight is an evolutionary adaptation consistent with a trade-off between the importance of their function and cost of their maintenance. It is also interesting to note that most studies of waterfowl (e.g. mottled duck *Anas fulvigula*, Fox et al. 2008; male mallards *Anas platyrhynchos*, Young and Boag 1982) report reductions in pectoral muscle size and yet no concomitant decrease in overall body mass as moult progresses, contradicting the reduced wing-loading hypothesis.

If the water body in which a duck is living dries down during the moult period, mortality is likely. It follows that over evolutionary time frames there must have been strong selective pressures for southern African ducks to (1) select permanent (and/or deeper) water bodies for moulting, even if food availability was low; and (2) minimize the duration of the flightless phase of moult.

The extent of phenotypic flexibility that exists in nomadic, south-temperate ducks during moult has never been explored. Indeed, there are few studies globally that address the issue of phenotypic flexibility of moulting waterfowl. black-necked grebes *Podiceps nigricollis* have small pectoral muscles during their flightless moult but during the two weeks prior to departure from the moulting grounds, their pectoral muscles double in size (Gaunt et al. 1990). To date, no such pattern has been documented for (more nomadic) southern African waterfowl. Douthwaite (1976) and Milstein (1993) both reported a decrease in body mass of flightless moulting red-billed teals *Anas erythrorhyncha* and seven captive Egyptian geese *Alopochen aegyptiaca* respectively, but could not link the change to phenotypic flexibility. Rather, they hypothesized that the mass decrease reflected the high energy costs of feather replacement and therefore expected it to continue as moult progressed.

In this paper we analyze physiological changes that occur during moult in Egyptian geese (Anatidae: a true duck, despite its common name *Alopochen aegyptiaca*). Specifically, we investigate whether or to what degree body condition, flight-feather growth, pectoral muscle mass and organ (gizzard, liver and heart) mass of Egyptian geese living in variable environments show phenotypic flexibility over the short period of flightless moult. We tested the hypothesis that moulting Egyptian geese adjust their mass and body condition to minimize the duration of the flightless period (when food availability is low). We expected that moulting ducks would lose weight, body condition, and pectoral muscle mass during moult; and that shortly before they regained the ability to fly, they would increase their pectoral muscle mass but would depart from their moulting grounds in poor overall body condition (Brown and Saunders 1998).

The study also evaluated two alternative hypotheses. The ‘feather growth cost hypothesis’, proposes that flight-feather moult is energetically costly to Egyptian geese and that this cost will be countered by building up body reserves before commencing moult. Once moult begins, birds are predicted to lose mass (including pectoral muscle mass) and body condition steadily during the moult period (Hanson 1962). The hypothesis does not predict significant change in liver and heart mass, but the gizzard is expected to decrease in mass with limited use (the cost-benefit hypothesis of Piersma and Lindström 1997). The second hypothesis was that remigial moult is not energetically costly to Egyptian geese. This hypothesis predicts that there will be no significant change in weight, body condition, organ (gizzard, liver and heart) mass or pectoral muscle mass during moult (Ankney 1979, Fox et al. 1998)

Materials and methods

Study sites

Adult Egyptian geese were caught at Barberspan Nature Reserve (26°33′S, 25°37′E) in the North West Province and Strandfontein Wastewater Treatment Works (34°05′S, 18°32′E) in the Western Cape Province, South Africa, between March 2007 and February 2008.

Barberspan Nature Reserve is a Ramsar-designated site in a semi-arid, summer rainfall region. It is a perennial pan that is shallow, alkaline and connected to the Hart’s River. When full, it covers an area of *ca* 2010 ha, but water levels fall considerably during late winter. Surrounding natural habitats comprise mainly gently undulating and flat, short to very short grassland interspersed with acacia trees. There are also surrounding farmlands used mainly for maize and sunflower cultivation. Large numbers of waterfowl and waders use the pan for foraging, breeding, roosting and moulting. In winter, Egyptian goose numbers regularly exceed 1000 individuals (Taylor et al. 1999).

Strandfontein Wastewater Treatment Works is in a coastal, winter-rainfall area. The site comprises 319 ha of open permanent ponds and canals, some with sandy islands, and 58 ha of terrestrial habitats, much of which is grassy. The woody species found in the area are mainly exotic *Acacia cyclops* (Kaletja-Summers et al. 2001). The hydrology of the site is managed and, depending on the management practice of the moment, some ponds have open mudflats that serve at roosting sites for waterfowl and feeding grounds for waders.

Fieldwork

Five hundred and seventy-two geese were caught during the study, 178 at Barberspan and 532 at Strandfontein, using maize-baited walk-in traps placed near the water’s edge. All geese were banded with metal rings and 410 individuals were also marked with a uniquely engraved colour ring. Mass was measured to the nearest gram using a spring balance. Moult, body condition (see below), forewing (to the tip of the longest primary), total head, culmen (to feathering) and tarsus length (from the end of the calcaneal ridge to the base of the complete scale before the toes diverge) were recorded for all birds. Head, tarsus and culmen lengths were measured with Vernier callipers to the nearest 0.1 mm.

Moult scores were recorded following de Beer et al. (2000). For each of the ten primary feathers moult was scored using a six point scale: 0 = fully grown old (worn) feather, 1 = feather missing or in pin, 2 = feather emerging from sheath up to 33% grown, 3 = new feather 33–67% grown, 4 = new feather from 67% to fully grown with remains of the sheath still visible, 5 = new, fully grown feather with no remaining traces of a sheath. Scores for each feather were summed, giving a total moult score. Because ducks undergo synchronous moult of the remiges, six distinct moult stages (i.e. 0, 10, 20, 30, 40 and 50) are recognizable.

A measure of body condition was derived from a body condition index (BCI) modified from Pehrsson (1987). The BCI was calculated by dividing body mass (g) by the product of two reliable structural measurements, *viz* tarsus and head length. Forewing length was not deemed a reliable structural measure because a) it may vary irrespective of body size (Pehrsson 1987), and b) most of the birds handled in this study were in moult. The BCI thus corrected mass for structural size.

Feather growth of captive birds

Forty adult moulting Egyptian geese (21 in June 2008 and 19 in March 2009) were caught at Barberspan Nature Reserve and housed for 5 days in a walk-in aviary (20 × 3 × 2.5 m). The geese were fed *ad lib.* on poultry grain (a mix of wheat, sorghum and crushed corn) and were free to graze the grass inside the aviary. Clean drinking water was provided daily.

The length (mm) of the right ninth primary flight feather (P9) was measured (from the point where the feather quill emerges out of the forewing to the tip of the feather) on the second day the geese were in captivity and again on the fifth day before they were released. P9 length measurements from 221 non-moulting Egyptian geese previously captured at Barberspan and Strandfontein during 2007 and 2008 were used to calculate the mean P9 length of non-moulting Egyptian geese. Assuming constant rates of change in the length of P9 (e.g van de Wetering and Cooke 2000) the average flightless duration and moult duration were established by dividing the mean P9 length of non-molting birds by the mean average P9 growth per day of flightless birds and the mean average P9 growth per day of all moulting birds respectively.

Muscles and organs

Fifteen birds, including three from each of the five moult categories, were collected from different areas around Cape Town. The birds were caught using walk-in traps and then euthanased in carbon monoxide chambers in compliance with Cape Nature permits and University of Cape Town animal ethics regulations. Pectoral muscles (*pectoralis major* and *supracoracoideus*) on both sides of the keel were cut from the sternum and at the underside of the humerus, excised and rinsed in distilled water. The gizzards were removed, emptied, and washed in distilled water. Organs were weighed (wet mass) using a laboratory scale with a 0.01 g precision.

Abdominal and mesenteric fat deposits were extracted and weighed. Subcutaneous fat was scored using Fox et al.'s (2008) six-point scale, where 0 = none; 1 = thin layer patchily distributed; 2 = thin layer evenly distributed, no blood vessels; 3 = medium layer, continuous over much of the body, obvious blood supply; 4 = thick layer continuous over body; and 5 = extensive thick deposits throughout, extending down the abdomen and around the neck.

Statistical analysis

A Student's unpaired *t*-test was used to test for significant differences in means of morphometric measurements from the two study sites. The Student's unpaired *t*-test was also used to test for differences in P9 growth rate between flightless and flight-capable moulting Egyptian geese. We calculated the mean P9 length of non-moulting geese and the mean P9 growth per day of moulting geese. We then fitted a linear regression model on all average P9 increments per day (mm) against initial P9 lengths and estimated the duration of moult and that of the flightless period. A correlation matrix was used to test for interaction terms between body mass, moult, pectoral muscle mass, organ mass and the body condition index. Regression models were fitted that best described changes in body mass, pectoral muscles mass, organ mass and body condition as a function of the moult stage. MANOVA was used to test for the effects of moult stage on weights of pectoral muscle, gizzard, liver and heart. Hypotheses were tested at the 5% level of significance and all analyses were carried out using the package SPSS 17 (2008).

Results

Mean head, culmen and tarsus lengths did not differ between Egyptian geese at Barberspan and Strandfontein. Non-moulting birds from Strandfontein were slightly, but not significantly heavier than those from Barberspan, with means of 2342 ± 49 g and 2166 ± 55 g respectively. Because there were no significant differences in the metrics of the birds from the two study sites, data were pooled for subsequent analysis.

Two hundred and forty-eight Egyptian geese with old feathers were caught at Strandfontein. Of these, 62 were moulting body feathers, mainly in the neck and back regions. No body-feather moult was observed on ducks in flight feather moult or on those that had just completed moult. No body moult was observed in all non-moulting Egyptian geese from Barberspan with both old and new flight feathers.

Mean body mass varied significantly ($F = 45.99$, $p < 0.001$) over the flightless moult period. Mass started to decrease as soon as the flight feathers were dropped and continued to do so until these flight feathers were at least two-thirds grown, after which mass started to increase (Fig. 1a). Mean BCI followed a similar trend to that of mass (Fig. 1b; $F = 71.17$, $p < 0.001$).

Feather growth of captive birds

Of the 40 birds held in captivity, 22 were flightless and 18 were capable of flight. The latter had a P9 length of at least 158 mm (two-thirds grown). The mean P9 growth rate for all the moulting geese was 4.59 ± 0.32 mm.d^{-1}. P9 growth rate decreased ($r = -0.56$; $p < 0.001$) as feathers elongated (Fig. 2). Mean feather growth rate of flightless birds was 5.54 ± 0.32 mm.d^{-1} and for birds capable of flight the equivalent growth rate was 3.52 ± 0.46 mm.d^{-1}. Therefore moulting geese are likely to be flightless for

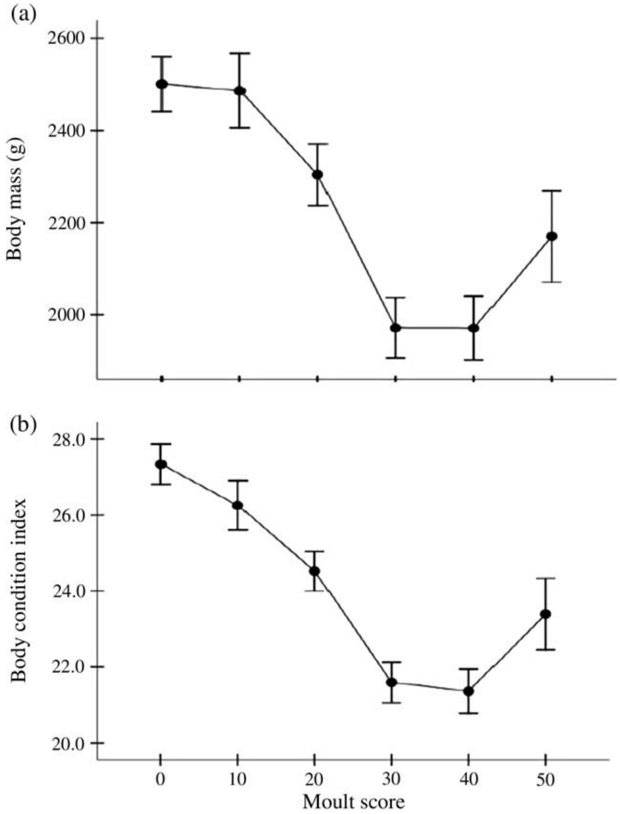

Figure 1. Mean mass (a) and body condition index (b) [± 95% C.L] as a function of moult score for Egyptian geese at Barberspan and Strandfontein.

at least 28.5 days. The mean P9 length of 221 non-moulting Egyptian geese was 247.22±1.31 mm. Therefore the average duration of moult based on the mean P9 growth rate for all moulting geese was 53.83 days, almost double the period of flightless moult.

Muscles and organs

Fat reserves with a score of 1 were recorded in three non-moulting geese and in one goose with new feathers. All other birds at all moult stages had no fat layer (score 0) and fat scores were not considered in subsequent analysis. The multivariate analysis suggests that masses of all organs measured, except the heart, varied significantly during moult (Table 1).

Pectoral muscle mass varied significantly during the moulting period. Non-moulting Egyptian geese with very old flight feathers (i.e. about to moult) had high pectoral muscle mass, accounting for at least 26% of total body mass. Once moult started, pectoral muscle mass decreased and continued to do so until the flight feathers were at least one-third grown, after which pectoral muscle mass started to increase (Fig. 3a). In other words, the regeneration of pectoral muscles during moult began before birds started to gain overall body mass.

Gizzard mass started to increase soon after the onset of moult, peaking when the flight feather were two-thirds grown, after which gizzard mass again decreased. Liver mass increased significantly ($p < 0.001$) as moult progressed, while heart mass remained unchanged (Fig. 4).

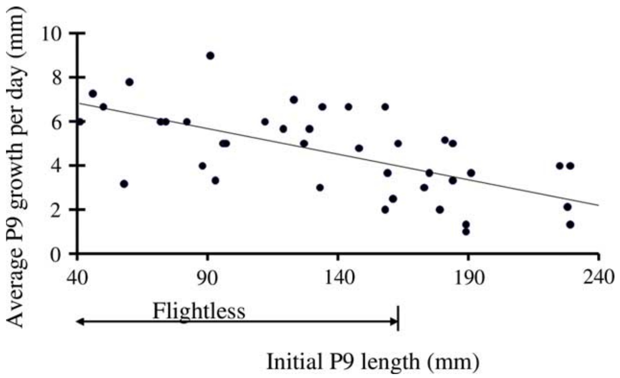

Figure 2. Average P9 increment per day (mm) as a function of initial P9 length for Egyptian geese in captivity. $Y = -0.023x + 7.778$; $r = -0.65$; $F = 28.75$; $p < 0.001$.

Discussion

Egyptian geese exhibit substantial phenotypic flexibility during moult. These changes in both muscle and organ mass can be interpreted as strategies to balance energy and nutrient demands with the costs associated with reduced food availability during the flightless moult period.

The overall loss of body condition and mass (Fig. 1) that occurs from the onset of moult until birds are once again able to fly, albeit not well, is interpreted as a response to a combination of lost foraging opportunity (reduced foraging time and low-quality food) and the cost of feather growth (Murphy 1996). At face value, these findings support the conclusions of Hanson (1962) and Folk et al. (1966) that moulting ducks are nutrient stressed and lose body mass and condition as a result. However, a logical extension of this interpretation is that geese will, periodically, starve to death during moult: there is apparently no such instance of this having been recorded (Halse 1984, Milstein 1993). An equally sound interpretation of this pattern is that geese reduce body condition and mass to a safe minimum level and then maintain this. Once flight feathers are more than two-thirds grown and the birds are able to fly, they can venture farther from water to higher quality foraging areas, allowing them to increase body mass and improve overall body condition towards the end of the moult period. Similar patterns of changing body mass during moult have been documented for north-temperate waterfowl (e.g. Ankney 1984, Jehl 1997). The most parsimonious explanation for changes in body mass and condition appears to be that they represent strategic responses which simultaneously reduce metabolic demand (through reduction in flight

Table 1. MANOVA of organ weights during moult

Organ Mass (g)	DF	F	p
Heart	5	0.266	0.923
Gizzard	5	127.957	<0.001
Liver	5	58.932	<0.001
Pectoral muscles	5	10.616	<0.001

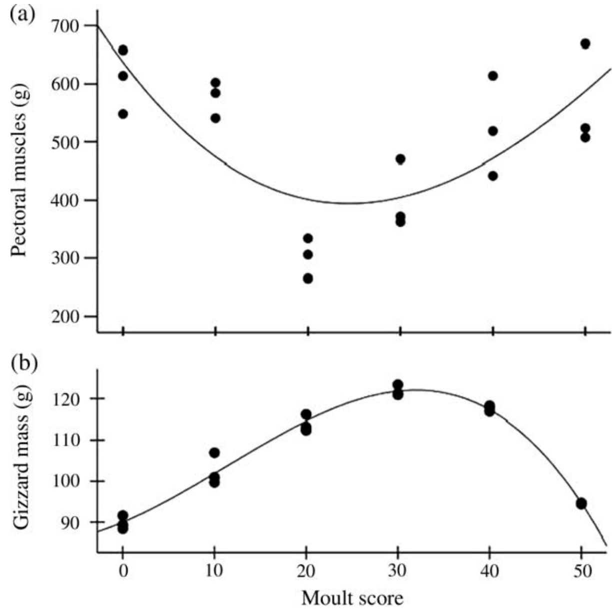

Figure 3. Changes in (a) wet pectoral muscle mass and (b) wet gizzard mass of Egyptian geese at Strandfontein as a function of moult score. Fitted polynomial curves: (a) $y = 0.3488x^2 - 18.165x + 630.63$; $r = 0.729$; $p = 0.003$, (b) $y = -0.001x^3 + 0.035x^2 + 0.94x + 89.98$; $r = 0.99$; $p < 0.001$

muscle mass – Fig. 3a) and wing loading (by reducing mass). The combined effect of this is to reduce the period of flightless moult (heavier birds would have to wait for longer before the feathers had grown sufficiently to enable them to fly). The advantage of shortening the flightless moult period is that it speeds up birds' access to higher quality foraging areas which in turn enables them to be in better condition at the end of moult than would be the case had they been flightless for longer. Predation pressure could be an alternative explanation for adopting a strategy to reduce body mass and shorten the flightless period (Owen and Ogilvie 1979, Sjöberg 1986). We were unable to test this hypothesis explicitly in our study. However, Egyptian geese probably have few natural predators in their southern African range: they are aggressive and are able to drive away large carnivores (Errington 1967, Howcroft 1968, Milstein 1993). In addition, they are only infrequently hunted for sport. This lack of extrinsic sources of mortality may contribute to explaining the species' major range expansion in southern Africa in the last 50–100 years (Okes et al. 2008).

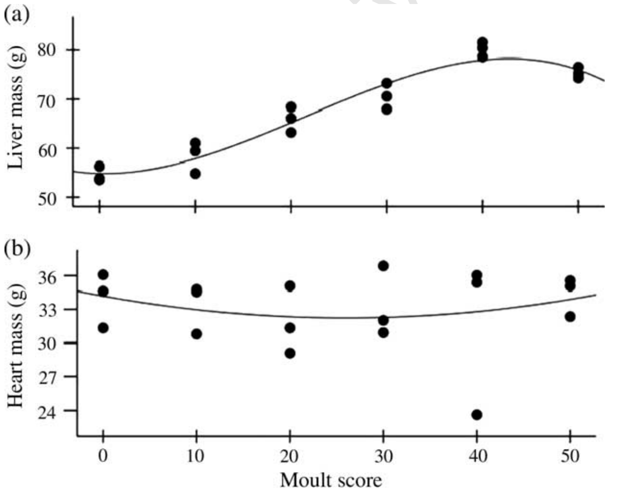

Figure 4. Changes in (a) wet liver mass and (b) wet heart mass of Egyptian geese at Strandfontein as a function of moult score. Fitted polynomial curves: (a) $y = -0.0063x^2 + 0.8102x + 53.013$ and $r = 0.9412$; $p < 0.0001$, (b) $y = 0.003x^2 - 0.15x + 34.188$ and $r = 0.229$; $p = 0.667$

The overall average daily primary feather growth rate of 4.59 ± 0.315 mm.day^{-1} for Egyptian geese in this study is less than that reported for most northern hemisphere ducks and geese (there are no southern African studies with which to compare these findings), although similar primary growth rates of 4.04 mm.d^{-1} have been reported for barrow's goldeneye *Bucephala islandica* (van de Wetering and Cooke 2000). The much larger mute swans *Cygnus olor* have an average primary growth rate of 6.5 mm.d^{-1} and barnacle geese *Branta leucopsis*, similar in size to Egyptian geese, have a primary growth rate of 7.5 mm.d^{-1} (Owen and Ogilvie 1979). Therefore Egyptian geese seem to take longer to complete their moult as compared to northern hemisphere waterfowl of equivalent size. However, the duration of the flightless period (*ca* 28–29 days) is relatively short compared to most northern hemisphere ducks (32–36 days; e.g Owen and Ogilvie 1979, Pehrsson 1987, van de Wetering and Cooke 2000). Despite their long moult duration, Egyptian geese are able to minimize the flightless period by reducing wing loading. They are able to fly with primaries that are only 67% grown as compared to northern hemisphere ducks (which maintain mass during moult) that can only fly once their primaries are at least 80% grown (Pehrsson 1987).

When flight feathers of Egyptian geese were half grown, and before flight was possible, the pectoral muscles gradually increased in size, allowing sustained flight once the flight feathers were over two-thirds grown. Similar atrophy and hypertrophy of pectoral muscles has been recorded for moulting greylag geese (Fox and Kahlert 2005) and black-necked grebes (Gaunt et al. 1990) in the northern hemisphere. These findings also concur with Piersma and Lindström (1997) who predict that peak flight performance is sustained by enlarged pectoral muscles whilst temporary flightlessness will cause the pectoral muscles to shrink. Ankney (1979) further suggests that the proteins and nutrients released as pectoral muscle atrophies may be used to supplement the growth of new flight feathers and the enlarged digestive organs.

The significant increase in the mass of the gizzard (which happens before the increase in pectoral muscle mass - Figs 3a, b) is interpreted as an adaptation to low diet quality during moult (i.e. below the point at which birds can maintain good body condition). Similar changes in gizzard size in response to diet have been demonstrated in domesticated Japanese quail *Coturnix japonica* (Starck 1999, Starck and Rahmaan 2003) and in red knots *Calidris canutus* (Dekinga et al. 2001). In both cases, gizzard mass increased within a week in response to hard-textured, low-protein forage and these changes reversed when birds were fed soft, high-quality food. The growth of the gizzard is a response to an increase in the work load involved in processing low-quality food (Starck 1999), as Egyptian geese are forced to do early in the moult period. In the case of Egyptian geese, gizzard mass started to decrease once birds were able to fly to higher quality foraging areas. This phenomenon has yet to be investigated for ducks in the northern hemisphere.

Our findings cannot fully discount the alternative hypothesis that the observed patterns are driven purely by the high energetic costs of feather growth. If the patterns were driven exclusively by stress (i.e. exogenously), it is possible to explain the pattern of increasing then decreasing gizzard mass as nothing more than a physiological response to forage quality (and one which has been demonstrated experimentally; see Starck 1999, Dekinga et al. 2001, Starck and Rahmaan 2003). It is also possible to explain why overall body mass decreases up to the time that birds are able to exploit high-quality food, because a combination of a poor nutritional environment coupled with the energetic demands of feather growth results in a negative energy balance (Murphy 1996). What cannot be explained by the 'feather growth cost' hypothesis is the allocation of increasingly scarce resources to pectoral muscles (i.e. increasing flight muscle mass while overall body mass continues to fall) before birds have access to high-quality food. This strongly implies an anticipation of need (i.e., a strategy) rather than an inevitable response to stress. Indeed, based on low-quality foraging conditions until the time the birds are able to fly, it is very difficult to understand how this pattern could be explained by the feather growth cost hypothesis. The findings of the study are consistent with the hypothesis that moulting Egyptian geese adjust their mass and body condition to minimize the flightless period; i.e. the phenotypic changes observed represent an adaptive strategy, *sensu stricto*.

The problem of reducing the flightless period is fundamental to all ducks that moult on water but graze on land. Grazing ducks in the northern hemisphere have an accelerated feather growth rate that they maintain by using stored fat reserves (e.g. Ankney and MacInnes 1978, Fox and Kahlert 2005). Unlike northern hemisphere ducks, Egyptian geese do not deposit fat reserves prior to moult and they have a slower feather growth rate. However, they trade off body mass, condition and muscle size for a reduction in wing loading that in turn shortens the flightless period.

In summary, unlike north-temperate ducks, moulting Egyptian geese in South Africa undergo substantial phenotypic changes during moult, including asynchronous changes (e.g. flight muscle mass increasing while body mass decreases). This phenotypic plasticity appears to have evolved to allow moult to be completed as rapidly as possible in environments characterized by stochasticity rather than predictability (which prevails at boreal latitudes). Specifically, the moult strategy of Egyptian geese is geared towards minimizing the duration of the flightless period and could not have been predicted based on prevailing northern hemisphere paradigms. These findings offer new insights into duck life history strategies in different environments, especially stochastic environments.

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Satellite telemetry of Afrotropical ducks: methodological details and assessment of success rates

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Despite widespread and increasing use of solar-powered satellite transmitters to tag wild birds, there are few published articles that detail how transmitters should be attached to different species and even fewer assessments of the overall field success of telemetry projects. The scarcity of this information makes it difficult to plan and budget for telemetry projects effectively. In this paper we present relevant information from a study involving a total of 47 individual ducks of two Afrotropical anatid species, Egyptian Goose, *Alopochen aegyptiaca,* and red-billed teal, *Anas erythrorhyncha,* using solar-powered GPS satellite transmitters of two different sizes (30 g and 22 g, respectively) at three very different southern African sites (Strandfontein wastewater treatment works in the Western Cape Province of South Africa, Barberspan Nature Reserve in the North West Province of South Africa, and Lake Manyame in north-central Zimbabwe). We present a full description of harness design and attachment and a survivorship analysis of the transmitters. Our results suggest that the 30 g units last longer than the 22 g units, with approximately 60% and 30%, respectively, of these PTTs (position tracking terminals) lasting longer than a year; 45% and 5%, respectively, lasting longer than two years; and 20% and 0%, respectively, lasting longer than three years. We strongly encourage the publication of comparable data sets so that future studies that rely on telemetry data can be planned with a realistic set of assumptions and limitations in mind.

Key words: **telemetry, Anatidae, survivorship, PTT, tracking, southern Africa.**

INTRODUCTION

As tracking technology has improved, the range of animals on which satellite tracking devices can be placed has expanded. Recent years have seen a growing number of people using satellite telemetry in unfamiliar contexts and with little prior experience. There are two critical areas in which more information is needed when starting to work with satellite telemetry. The first is during the planning stage, where estimating likely success rates and budgeting for different aspects of the project must often be done before direct personal experience of satellite telemetry has been obtained. The second concerns the finer details of animal capture and transmitter attachment.

On the first need, there is relatively little independent (i.e. non-manufacturer-provided), quantitative, peer-reviewed information available about success rates of studies using transmitters and solar-powered satellite transmitters in particular. Some exceptions exist; for example, Britten *et al.* (1999) gave a detailed analysis of deployment success of 30 g satellite transmitters on Peregrine Falcons *Falco peregrinus,* and Roshier & Asmus (2009) described a harness design used to attach satellite tags on two small-bodied waterfowl, the grey teal *Anas gracilis* and the wandering whistling-duck *Dendrocygna arcuta,* in Australia.

Effective planning of transmitter-based studies relies heavily on quantitative field information. Although the number of transmitters that should be deployed on a given population to provide a meaningful estimate of movement patterns has been analysed quantitatively using simulation models (e.g. Lindberg & Walker 2007), such studies do not indicate the actual number of transmitters that must be purchased because the total number will be what is required in terms of the science plus additional transmitters to compensate for attrition in the field. Attrition rates should ideally be made available for different taxa, particularly where the devices concerned are relatively expensive, so that they can be factored into decisions about whether or not to undertake satellite tracking studies in the first place.

On the second need, while informal networks and manufacturer-supplied information can meet information demands to some extent, it is still difficult to find detailed explanations of field attachment techniques. Relatively few published,

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*Author for correspondence. E-mail: graeme.cumming@uct.ac.za peer-reviewed papers (three exceptions being Malecki *et al.* 2001; Miller *et al.* 2005; and Roshier & Asmus 2009) have explained methods of satellite transmitter attachment to waterbirds in sufficient detail to permit imitation. There are a few published descriptions of methods for attaching short-duration battery-powered transmitters that work for less than a month (Gudmundsson *et al.* 1995; Guicking *et al.* 2001) but we were unable to locate any publications dealing directly and in depth with methods for attaching long-duration solar-powered GPS transmitters to waterbirds.

In this paper we present (1) a proven, effective approach to transmitter attachment on both small-bodied and medium-sized ducks; and (2) an assessment of transmitter performance under the relatively hot, dry field conditions of southern Africa. We envisage that this information will be useful to other field ecologists in both the planning and the deployment stages of research on waterbirds. Our analysis is based on data from the 44 satellite GPS transmitters that were deployed on two species of African duck, Egyptian geese (*Alopochen aegyptiacus*) and red-billed teals (*Anas erythrorhyncha*), over the period between January 2008 and May 2011.

METHODS

Background

The transmitters were deployed in a study that focused on understanding the movement patterns of potential vectors of avian influenza. The number of transmitters that we deployed was limited by available funding to a total of 44 units. We decided *a priori* to study two species rather than one, so that we could gain insights into anatid ecology by comparing their movement patterns; to include two different foraging styles (dabbling and grazing); and to study birds that could be found across southern Africa in high abundances. We also wanted to study individuals that were using the study sites that formed part of the broader research programme on avian influenza (see Cumming *et al.* 2011 for full details). An initial risk assessment exercise (Cumming *et al.* 2008) highlighted red-billed teal and Egyptian geese as two of the highest risk species for influenza transmission. We tagged birds of each species in roughly equal numbers (at least seven per site was our original goal, with the underlying aim of getting at least $n = 5$ workable data sets per species and site) at our three core field sites. These sites were deliberately selected along a latitudinal gradient to maximize our chances of learning about the responses of birds and their pathogens to variations in climate, wetland distributions, and proximity to northern hemisphere populations. We had no prior data on PTT (position tracking terminal) survival and simply hoped that the sample size would be sufficient.

Study sites and capture methods

We captured and tagged birds at three different sites along a latitudinal gradient: Strandfontein wastewater treatment works (34°05′S, 18°32′E) in the Western Cape Province of South Africa, Barberspan Nature Reserve (26°33′S, 25°37′E) in the North West Province of South Africa, and Lake Manyame (17°49′S, 30°36′E) in north-central Zimbabwe (Fig. 1). These areas represent three quite different environments, and hence provide a good test of both the transmitters themselves and the attachment approach. Birds were captured using a cannon net, maize-baited walk-in traps placed near the water's edge, and mist nets placed across narrow (10–40 m wide) inlet channels.

The numbers of birds tracked per study site are summarized in Table 1. We monitored the progress of the transmitters via the Argos web interface (https://argos-system.clsamerica.com/cwi/Logon.do), periodically checking the GPS coordinates. Where coordinates were in the same location through the day for more than about three days, we assumed that either the transmitter had fallen off or the bird had died. In these instances we visited the site (where feasible) to see if we could recover the transmitter.

Transmitter details

The general principle in telemetry studies is to use the largest transmitter that the bird is comfortable with and still weighs less than five percent of the bird's mass (Caccamise & Hedin 1985), because reductions in transmitter size occur in parallel with reductions in transmitter storage capacity and functionality. For example, satellite GPS capability at the time when we started our study was not available in solar-powered transmitters under the weight of 22 g; smaller transmitters provided satellite triangulation (Argos) fixes, but these are considerably less accurate than GPS data.

We used solar-powered satellite GPS transmitters manufactured by Microwave Telemetry (http://www.microwavetelemetry.com). All transmitters were deployed on birds between 15 January 2008 and 17 January 2009 (see details in Table 1). We

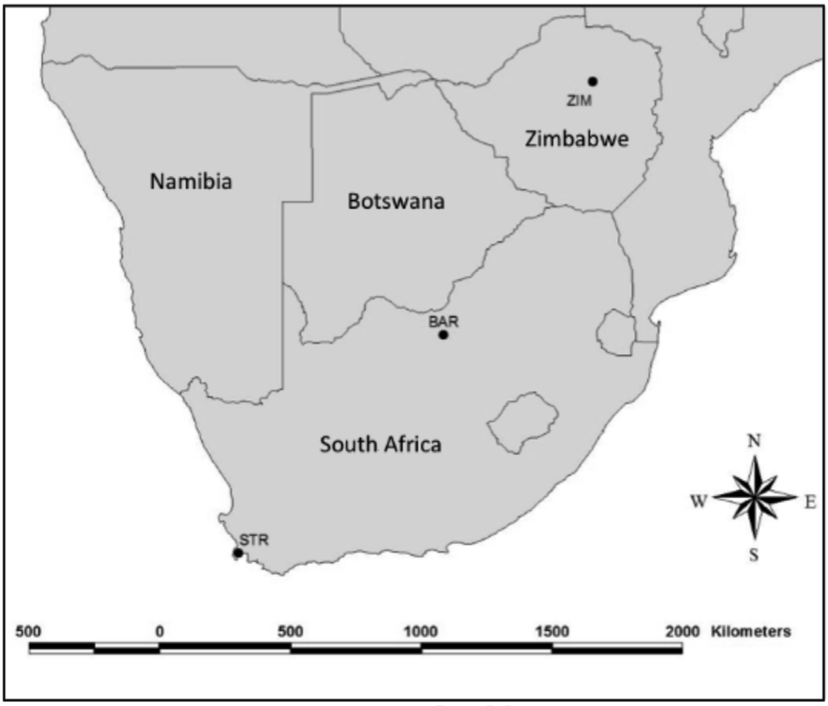

Fig. 1. Locations of our tagging sites in southern Africa: Strandfontein (STR), Barberspan (BAR), and Lake Manyame (ZIM).

used 22 g transmitters for red-billed teal (mean adult weight 642.24 g, S.D. 97.33 g) and 30 g transmitters for Egyptian geese (mean adult weight 2299 g, S.D. 432.46 g). The larger transmitters are capable of storing more power than the smaller transmitters and hence can generate more fixes per day. The transmitters were set to capture data every two hours (30 g PTTs) and every four hours (22 g PTTs), respectively. We did not request separate summer and winter programmes because changes in day length are relatively small at most of our study sites. Since we did not need real-time ('instant') data, and since sending data to a satellite is energy-intensive for a small solar-powered unit, we used the manufacturer's option to set the unit to store and upload data at regular intervals.

Table 1. Summary statistics for PTT characteristics and performance during the study. The number of birds carrying PTTs differs from the number of PTTs deployed because we were able to recover and redeploy five PTTs (two are not included in these results because they are yet to be redeployed).

Characteristic or performance statistic	Egyptian geese	Red-billed teal
PTT weight	30g	22g
PTT sampling frequency	Every 2 h	Every 4 h
Number of individual PTTs deployed	22	22
Number of different birds carrying PTTs	25	22
Mean number of days obtained from PTT over study period	>482 (4 PTTs still operating)	243
Mean number of resightings obtained per PTT per day	7.4 out of a possible 12	5.1 out of a possible 6
Number of immediate (<2 days) failures	1	1
Successful recoveries	5	0
Approximate survivorship after 1 year	60%	30%
Approximate survivorship after 2 years	45%	5%
Approximate survivorship after 3 years	20%	0%

The larger transmitters were set to send data to the satellite every three days, while the smaller transmitters were queried every five days. Note that these transmitters provide both the standard Argos location data, with typical accuracies around 1 km, and a high-quality GPS fix that is accurate to within 20 m. Although various approaches for picking high-quality data from Argos data sets are available (Austin *et al.* 2003; Jonsen *et al.* 2003; Jonsen *et al.* 2005), the GPS data were of far higher quality than the Argos data and consequently we used only the GPS data in subsequent analyses.

Attachment details

Implantation is not a sensible option for a solar-powered transmitter. We used a backpack harness design based partly on details given by Smith & Gilbert (1981) but relying heavily on personal communications from Peter Frederick (Department of Wildlife Ecology and Conservation, University of Florida), who has used satellite telemetry extensively on wood storks. We tested our attachment methods on five captive birds of each of our study species using wooden model transmitters with wire 'antennae'. The test birds were wild-caught from our study sites, held in shadecloth cages, and observed regularly for two days before release.

Individuals for telemetry were selected from much larger numbers of birds caught (see Cumming *et al.* 2011). We only used birds that appeared to be in good condition, that were of average or larger than average size, and that had few or no feather lice (this last condition was introduced because lice might focus on the area under the transmitter straps, creating additional discomfort). The approach outlined below takes 15–20 minutes per bird (in contrast to the 45–60 minutes reported by Britten *et al.* (1999), although ducks do tend to be more docile than raptors).

The first step in transmitter attachment was to glue an extra rubber pad underneath the transmitter to further raise it (i.e. bringing the total number of pads up to two, the pad provided by the manufacturer and our own addition). We began doing this after noticing that transmitters appeared to be getting covered by back feathers on Egyptian geese. All five of the transmitters to which an extra pad was fitted functioned well for over a year.

The straps of the backpack were made from teflon ribbon (Telonics Inc., U.S.A.). The ribbon was measured and cut individually to fit each bird. The PTTs have a single metal guide loop at the front and two metal guides (placed laterally, opposite one another) at the back. Having measured and cut two strands, we attached one strand to the front of the transmitter and one to the back. We looped the ribbon around through itself in the front to make a lark's head knot (see Fig. 2) and at the back, double-looped it through the metal guide. At this stage it would have been possible to glue or sew the Teflon together around the metal loops of the PTT to prevent any movement of the transmitter along the straps, but we opted not to do so for two reasons: (1) to allow the bird freedom in settling the backpack in a comfortable spot; and (2) in case glue or thread would weaken the straps. None of our recaptured or resighted transmitters with intact straps showed any sign of the transmitter having shifted from its desired position.

Our two study species were of different sizes and the larger Egyptian geese showed considerably more variation in body size than the red-billed teal. Red-billed teal strap lengths (i.e. total length of ribbon needed to make the strap) were anterior, 300 mm (S.D. 9 mm, $n = 22$) and posterior, 310 mm (S.D. 20 mm, $n = 22$). Egyptian Goose strap lengths were anterior, 373 mm (S.D. 53 mm, $n = 25$) and posterior, 439 mm (S.D. 34 mm, $n = 25$). 'Safe' estimates of required ribbon length for purchasing purposes would use (mean + S.D.) of anterior and posterior straps for each PTT that is to be deployed. We placed transmitters on more Egyptian geese than red-billed teal because we recovered five 30 g transmitters and deployed three of these on new individuals.

Once the straps were attached to the transmitter it was placed in the centre of the bird's back, between the wings, and the ends of the posterior strap were passed under the flank feathers and around to the ventral surface anterior to the legs. The bird was then turned onto its back with one hand while the posterior loops were pinned together with the other hand. The same procedure was repeated with the ends of the anterior strap. Once both anterior and posterior straps had been pinned in position separately, we pulled the straps together and pinned them to one another (Fig. 2).

At this stage the harness should fit snugly with a cross over the breast of the bird and space for two fingers underneath the straps. It is important when tightening the straps to pass the anterior strap over the anterior end of the wing bone (which can be felt beneath the skin) and to ensure that the posterior strap is far back enough that it

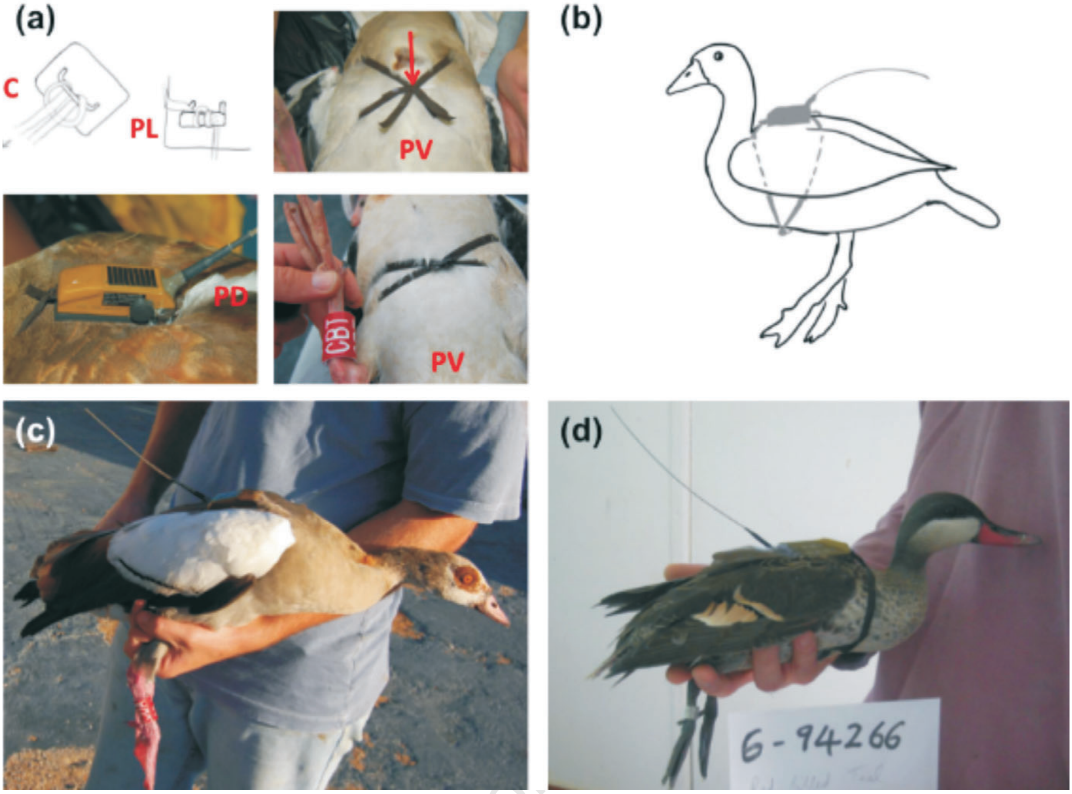

Fig. 2. Illustration of attachment methods as described in text. **a**, The transmitter is held in place by two teflon loops that are sewed together on the breast (the arrow in the top right panel indicates the sewing point; P indicates the posterior end of the bird; D, dorsal; V, ventral; L, lateral; and C, a cranial view of the anterior end of the PTT); **b**, the transmitter sits comfortably on the back with loops passing under the front and back of the wing; **c**, attached 30 g transmitter on an egyptian goose; **d**, attached 22 g transmitter on a red-billed teal. Note the appearance of a looser front loop on the smaller, less padded bird (this loop will settle more tightly as the bird flies).

will not rub on the trailing edge of the wing. With moulting birds that are in poor condition it is best to make the harness a little looser than the two-finger rule implies, so that the bird can move easily when it returns to normal condition. The straps will in any case sink into the feathers, particularly on a downy bird like a duck, and the bird will shift the transmitter into a suitable position as it flies. The arrangement can be tightened or adjusted at this point by repinning anterior and posteriors traps individually until the transmitter sits comfortably in position.

Once the straps were pinned in place, we sewed them together at a single point on the breast (Fig. 2). We used upholstery thread and a thick needle for the sewing. Thread was fastened onto the lower strap and passed through all four straps on each pass of the needle, much like sewing on a button. The pins were removed mid-way through the sewing process and we ended the thread on the strand closest to the bird's body, this being less likely to rub on vegetation. The motivation for using thread and a single attachment point is humane; the thread should last as long as the PTT, but should perish before the ribbon. When the thread perishes, all four straps should loosen and fall off at roughly the same time, preventing the bird from having to drag a half-attached transmitter around for long periods. Given that wild Egyptian geese in southern African have been shown to live for up to 15 years (Underhill *et al.* 1999) it is important (as discussed above) that transmitter loss can occur easily and without harm to the animal.

Although the use of metal crimps leads to slightly faster processing times (6–7 minutes, according to Roshier & Asmus 2009), there is no evidence to suggest that an additional 7–8 minutes of handling time has any long-term impact on the bird's subsequent performance or survival. We decided not to use crimps for two reasons. The first is that Egyptian geese in particular spend substantial amounts of time in or near brackish and salty water, which could lead to rapid rusting of metals and potentially harmful broken edges. Second, the crimping method (Roshier & Asmus 2009) lacks built-in redundancy, meaning that if the harness straps do not wear and eventually tear, a relatively long-lived bird may be stuck with a non-functional transmitter for the remainder of its natural life; and if one crimp comes undone, or one strap tears, the bird is left with a debilitating swinging burden. We prefer instead the option of sewing harness ends together with cotton thread at a single attachment point, which seems more likely to result in a uniform release of all straps when the thread decays.

When working with teflon ribbon it is necessary after sewing to superglue the ends of the ribbon before cutting them short (melting them with a flame was suggested by one reviewer). We would typically saturate about 5 mm of ribbon with glue and cut the thread in the middle of the saturated area. Teflon ribbon unravels easily and if the end looks messy and is too short to cut cleanly or glue effectively, it is better to start the whole process again. Sharp scissors are important to cut the teflon cleanly and without leaving trailing threads. We also placed a drop of superglue over the thread itself to reduce immediate wear and tear.

After the PTT had been attached, the thread cut and the long ends of the ribbon glued and trimmed, two important steps remained. The first of these was to trim the back feathers, particularly on the larger birds. We cut feathers extensively around the transmitters to remove any back feathers that might potentially be preened over the solar panel of the transmitter.

The second step was to remove the magnet from the transmitter to start the PTT running. According to the manufacturer, the PTT searches more intensively for satellites after it is started, using more charge; keeping the PTT in direct sunlight during this initial period should ensure that the capacitor does not drain. Magnet removal is therefore best done in the morning, in sunlight, and we generally held the bird stationary for 5–10 minutes with the sun fully on the solar panel after removing the magnet. The issue here is not whether the PTT is fully charged prior to attachment (it obviously should be) but rather, that it is important to ensure that the PTT can recharge during and just after the battery-heavy period when it locates satellites. Another slightly lower-stress option for the bird would be to start the PTT running (by removing the magnet) 10 minutes prior to attachment, leaving the PTT in direct sunlight during this period.

RESULTS

The overall performance of the transmitters to date is summarized in Table 2 and Fig. 3. As these summaries show, there were substantial performance differences between the two transmitters used in the study, with the 30 g PTTs outperforming the 22 g PTTs in most respects.

The average numbers of resightings per day indicate that not all PTTs provided a full set of readings each day. In some cases PTTs transmitted erratically and then resumed normal transmission; and in others, erratic transmission preceded transmission termination. Given that contact with satellites is not an issue at most of our study sites, there are four primary reasons why a transmitter might fail to transmit for periods of two weeks or more, including: (1) loss of the transmitter; (2) mortality of the bird; (3) failure of the solar panel to recharge, for instance if the bird is nesting under a bush or preening feathers over the solar panel; and (4) technological problems with the transmitter. In practice it is difficult to distinguish between these different kinds of transmitter failure unless the PTT is re-sighted or recovered, so our overall estimate of expected PTT performance in the field should not be interpreted as any kind of indictment of the manufacturer.

We obtained evidence of causes of failure for six of the 38 transmitters that had failed by the time of writing. One was recovered from outside a jackal den at Leeuwpan (near Barberspan), and a second from a bird that had died in a floating bed of water hyacinth on Lake Manyame. A colleague sent us a photograph of a third Egyptian goose carrying a transmitter that had stopped sending data but still looked perfectly attached and had its solar panel visible, suggesting a technological failure; and a fourth seemed fine in testing but developed a broken clock within hours of release. A fifth PTT was returned from an Egyptian Goose that had been shot in Somerset West, near Cape Town. The sixth was found still working on the Steenberg Golf course in Tokai, Cape Town, with the harness worn through on the ventral side about 4 cm above the point where the four strands met. We could thus attribute three failures to bird mortality,

Table 2. Details of species, transmitters, and sample sizes for each individual data set used in this analysis. Column headings are as follows: ID, transmitter ID; Wt, transmitter weight; Fq, Sampling frequency in hours; Site, location at which transmitter was attached; Start, Starting Date; End, Ending date; Days, total period over which transmitter was active; Fixes, number of reliable locations obtained; Distance, total distance in km moved by the animal during the study period (i.e. the sum of all line lengths between successive points); and MCPkm^2, area of a Minimum Convex Polygon around all points, in square kilometres. Species codes are: RBT, red-billed teal, *Anas erythrorhyncha*; EG, Egyptian goose, *Alopochen aegyptiaca.* Site codes are: STR, Strandfontein; BAR, Barberspan; MAN, Lake Manyame; ZIM, Zimbabwe. Note that the data that we have analysed for the purposes of this table include readings until the end of October, 2010; the repeated end values for 31 October reflect transmitters that were still active at that time rather than a mass mortality event. In addition, two PTTS that did not provide sufficient data to analyse are excluded from the table.

ID	Wt	Fq	Species	Site	Start	End	Days	Fixes	Distance
77092	22	4 h	RBT	STR	13 Mar 08	27 Mar 09	379	1 800	2 442.302
77093	22	4 h	RBT	STR	13 Mar 08	07 Sep 08	178	990	676.02
77094	30	2 h	EG	STR	15 Jan 08	04 Sep 09	598	4 066	1 731.27
77095	30	2 h	EG	STR	15 Jan 08	12 Dec 09	697	3 399	1 980.71
77096	22	4 h	RBT	STR	13 Mar 08	22 Mar 08	9	47	9.40
77097	22	4 h	RBT	STR	14 Mar 08	14 Jun 08	92	404	904.22
77098	22	4 h	RBT	STR	14 Mar 08	25 Nov 09	621	3 550	2 338.70
77099	22	4 h	RBT	STR	14 Mar 08	16 May 09	428	1 859	1 711.72
77100	22	4 h	RBT	STR	14 Mar 08	15 Jun 09	458	2 077	1 605.18
77101	22	4 h	RBT	BAR	09 Apr 08	06 Jan 09	272	800	921.20
77102	22	4 h	RBT	BAR	10 Apr 08	21 Apr 10	741	4 155	4 376.74
77103	22	4 h	RBT	MAN	05 May 08	24 Aug 08	111	610	1 701.39
77104	22	4 h	RBT	MAN	05 May 08	26 Jan 09	266	1 431	2 432.45
77105	22	4 h	RBT	MAN	05 May 08	14 Jun 08	40	198	89.87
77106	22	4 h	RBT	MAN	06 May 08	26 Jul 09	446	2 587	2 208.60
77107	22	4 h	RBT	MAN	06 May 08	26 May 08	20	83	149.65
77108	22	4 h	RBT	MAN	06 May 08	29 Aug 08	115	644	734.63
77109	22	4 h	RBT	MAN	07 May 08	31 Oct 10	232	1 307	1 290.51
77110	22	4 h	RBT	MAN	07 May 08	06 Jun 08	30	163	223.74
77111	22	4 h	RBT	BAR	25 Jul 08	06 Jan 09	165	701	283.74
77112	22	4 h	RBT	BAR	07 Jun 08	21 May 09	348	1 843	1 773.68
77113	22	4 h	RBT	BAR	26 Jul 08	18 Sep 08	54	210	280.72
77115	22	4 h	RBT	BAR	11 Oct 08	04 Sep 09	328	1 538	1701.65
77116	30	2 h	EG	STR	16 Jan 08	01 Apr 08	76	803	216.05
77117	30	2 h	EG	STR	19 Jan 08	14 Mar 08	55	148	22.33
77118.1	30	2 h	EG	STR	17 Jan 08	15 Jan 09	364	483	1 054.51
77118.2	30	2 h	EG	STR	17 Jan 09	31 Oct 10	752	6 012	4 415.52
77119	30	2 h	EG	STR	17 Jan 08	16 Jun 08	151	805	646.28
77121	30	2 h	EG	STR	18 Jan 08	15 Apr 08	88	284	31.48
77122.1	30	2 h	EG	BAR	09 Apr 08	15 Apr 08	6	73	17.10
77122.2	30	2 h	EG	BAR	07 Jun 08	06 Mar 10	637	2 861	4 810.19
77123.1	30	2 h	EG	MAN	07 May 08	16 May 08	9	110	249.16
77123.2	30	2 h	EG	STR	04 Dec 08	29 Nov 09	360	1 894	731.03
77124	30	2 h	EG	MAN	07 May 08	21 Jul 08	75	463	525.86
77125	30	2 h	EG	MAN	07 May 08	31 Oct 10	1007	8 035	13 645.93
77126	30	2 h	EG	MAN	07 May 08	09 Jan 09	247	2 682	2 403.61
77127	30	2 h	EG	BAR	07 Jun 08	31 Oct 10	976	6 401	6 381.43
77128	30	2 h	EG	BAR	07 Jun 08	31 Oct 10	976	6 323	7 386.85
77129	30	2 h	EG	BAR	07 Jun 08	19 May 09	346	3 491	5 662.69
77130	30	2 h	EG	BAR	07 Jun 08	31 Oct 10	976	5 018	9 631.07
77131	30	2 h	EG	BAR	07 Jun 08	13 Oct 09	493	1 209	262.47
77132	30	2 h	EG	BAR	07 Jun 08	31 Oct 10	976	4 960	6 471.34
77133	30	2 h	EG	STR	04 Dec 08	24 Nov 09	355	2 534	2 075.05
77134	30	2 h	EG	STR	04 Dec 08	31 Oct 10	796	5 400	2 079.07
77135	30	2 h	EG	STR	04 Dec 08	31 Oct 10	796	6 755	4 811.54

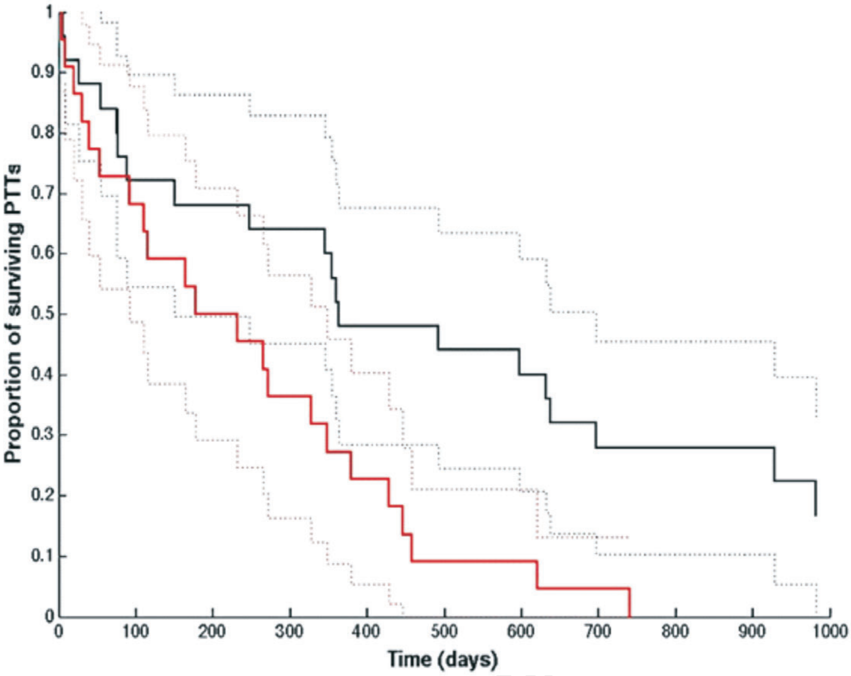

Fig. 3. Survivorship curve showing proportion of active transmitters against time for 30 g transmitters on Egyptian geese (upper, black curve) and 22 g transmitters on red-billed teal (lower curve). The dotted lines indicate 95% confidence intervals in each case. The upper curve does not intersect with the *X*-axis because four of the 30 g PTTs were still active at the time of writing.

two to technological failures, and one to a damaged harness.

Several other transmitters sent data patchily, with frequent low charge readings, suggesting that these were suffering from coverage of the panel by feathers. In some cases these problems seem to have resolved themselves, while in others the transmitters eventually stopped sending signals.

Tagged birds routinely flew on release. Evidence suggesting that the transmitters did not greatly affect the behaviours or survivorship of the birds is provided by (1) the distances moved by some individuals (as far as 950 km in two days, in the case of one Egyptian goose); (2) observations on a tagged individual that successfully bred and raised six chicks on the Cape Peninsula, near Cape Point; and (3) the persistence of some transmitters on far-moving birds for over three years.

DISCUSSION

Based on various informal discussions with colleagues who have worked on ducks, we had a relatively high overall success rate with satellite transmitters on this project (Fig. 3). Our success rates for solar-powered GPS transmitters still functioning after one, two and three years, respectively, is much higher than that of Miller *et al.* (2005), who tagged more than a hundred pintails with transmitters and had less than 17% success after a year. Our overall success levels are difficult to compare to other studies because most of the long-duration (over a year) telemetry studies on water birds have relatively small sample sizes (e.g. Izhaki *et al.* 2002; Cappelle *et al.* 2011), and most studies with broader sample sizes (e.g. Gaidet *et al.* 2010) have not reported transmitter success rates or survivorship curves. The study of Roshier & Asmus (2009) is currently the only study that we can compare our transmitter success rate with. They tagged 23 grey teal and 22 wandering whistling-duck and had 30% and 14% success rate after a year, respectively. We had an identical success rate on teal after a year, but our Egyptian Goose tag success rate of 60% far exceeds theirs. We do not have sufficient data from other species to know whether this difference is due solely to transmitter size or to some other aspect of the bird's life history or foraging behaviour.

Another common concern in tracking studies is the influence of the harness on thermoregulation and preening rates, respectively. In the southern African climate the influence of breast straps on feather insulation appears to be negligible. Egyptian geese, for example, are winter breeders that avoid excessively high temperatures; their primary problem is getting cool, rather than getting warm, and they use their long, featherless legs as thermal windows. We did not observe tagged birds preening their breast feathers at any point during the project, although on a few occasions they were observed preening their back feathers for unusually long periods immediately following attachment (as might be expected from some individuals when using a backpack harness of any sort). The successful breeding attempt of one of our tagged birds further supports the idea that the transmitters did not modify the animals' behaviour in any significant way.

Solar-powered GPS transmitters are well-suited to studies of ducks because anatids are large enough to carry transmitters, long-lived (Underhill *et al.* 1999), and spend much of the day in direct sunlight. They also move sufficiently far that other, cheaper telemetry methods (such as VHF transmitters) are unfeasible. We would expect success rates to be lower with water birds that have longer back feathers (e.g. flamingos and herons) and that spend more time in the shade (e.g. snipe and rails), although raising the PTTs on additional neoprene pads may make tracking of these species more viable.

Given the budgetary constraints faced by most studies, transmitter failures or bird mortalities (with resulting transmitter loss) can influence the overall scientific output from a project and potentially tip the balance between including or excluding telemetry. This is particularly true when only a small number of transmitters can be afforded; hypothetically, an anticipated 40% success rate for a given species–PTT combination over a year may make the purchase of 10 transmitters scientifically meaningless except as a pilot project, while an anticipated 80% success rate would yield twice the sample size. Similarly, for small projects where it is hoped that one or two transmitters will provide useful information, there is no guarantee that those transmitters will work. Total numbers of transmitters must thus be carefully assessed in advance, using likely declines in transmitter numbers over time as a key reference point. Information on the success rates that can be expected is highly valuable; we wish that it had been available when we were preparing and budgeting for this project. In our own case, if we had seen the survivorship curves in Fig. 3 prior to undertaking the study, we might have either (1) purchased only the larger PTTs and focused on another larger duck species (such as spurwing goose, South African shelduck, or comb duck) rather than red-billed teal; or (2) reduced the number of sites at which we attached PTTs to red-billed teal.

Transmitter survivorship curves offer a strong starting point for assessing how many transmitters would be needed to study the movements of other duck populations in southern Africa, and we would strongly advocate their being routinely published as a component of tracking studies.

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Responses of an African wading bird community to resource pulses are related to foraging guild and food-web position

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SUMMARY

1. The immediate impacts of nutrient inputs on aquatic systems are well documented, but the ways in which resource pulses affect the wider food web of water-associated vertebrates remain obscure.
2. We monitored the wading bird community of Barberspan, a natural freshwater lake and Ramsar wetland in South Africa, before, during and after the addition of a pulse of nutrients in the form of a sewage overspill from an upstream processing facility. We counted waders at 13 points around the lake over 3 years, every 2 months from March 2007 to March 2010, and sampled water quality during all counting periods from January 2008 to January 2010.
3. We used our data to test the hypothesis that wading birds that forage directly at lower trophic levels and/or on prey populations that have fast turnover rates, such as those of phytoplankton and invertebrates, will be more heavily influenced by nutrient addition than birds that forage on species with lower turnover rates and/or at higher trophic levels (such as frogs and fish).
4. During the sampling period Barberspan experienced a significant, nutrient-driven decline and subsequent recovery in dissolved oxygen and pH. This trend was mirrored by significant changes in the wading bird community. Partial Mantel tests and Canonical Correspondence Analysis (CCA) showed that the nutrient pulse had marked short-term, negative impacts on both the diversity and the abundance of medium-sized, shoreline foragers such as scolopacids (e.g. sandpipers).
5. Our analysis supports the proposal that both food-web position and the turnover rate of the prey population are strong influences on ecological responses to resource pulses. Analysis of time series of principal components that describe community composition suggested that recovery of the prey base was rapid and that the bird community was able to respond via immigration. These results must, of course, be considered provisional in the absence of replicated experimental data.
6. More generally, we interpret our results as suggesting that two different mechanisms act in different directions to determine the sensitivity of secondary and tertiary consumers to changes in their prey. First, for 'earlier' consumers (i.e. that forage lower in the food web), there are (on average) fewer generalist consumers and fewer stored nutrients in the intervening trophic levels. This increases the sensitivity of earlier consumer populations to changes in the composition of the primary consumer community. Second, the dynamics of prey populations lower in the food web tend to be faster, making recovery faster and serving to decrease the sensitivity of earlier consumers to perturbations. These dynamics may obscure the impacts of nutrient pulses in cases where additional analysis of system trajectories is not undertaken.

Keywords: Barberspan, eutrophication, nutrients, resilience, shorebird

Introduction

The sensitivity of aquatic food webs to the addition of nutrients such as phosphorus and nitrogen is well documented. Shallow lakes, in particular, are capable of existing in alternate stable states (clear and turbid) in which each state is maintained by a set of biotic and biophysical feedbacks, mediated by phosphorus levels in

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the water and lake sediments (Carpenter, 2003; Scheffer & Jeppesen, 2007; Scheffer & Van Nes, 2007). These different states are accompanied by marked and relatively well-documented (at least in cold northern hemisphere lakes) changes in the composition of the communities of plants and animals that are present. However, the community-level implications of nutrient additions and resulting changes in the aquatic community for other, less strictly aquatic food web components that use the same body of water – such as birds and mammals – have not been widely explored.

Nutrient inputs of high magnitude that occur from a point source over a finite time period are a form of resource pulse. The study of resource pulses under a unifying theoretical framework is a relatively new field of research in ecology (Yang *et al.*, 2010). It is expected to contribute to the development of ecological theory in two main ways: (i) through the identification of shared ecological mechanisms underlying the responses of different ecosystems to resource pulses and (ii) through the opportunities that resource pulses provide for the exploration and testing of other generalities in ecology, including (for example) ideas about the propagation of indirect effects in food webs, apparent competition, differences between aquatic and terrestrial systems, system resilience, and resource–consumer theory (Holt, 2008; Yang *et al.*, 2008, 2010).

In this study, we explore the consequences of a single resource pulse (an input of partly treated sewage that occurred when the banks of an upstream facility were flooded) for the wading bird community of Barberspan, a Ramsar-listed wetland and protected area in north-west South Africa. Given the diversity of diets and foraging styles within the wading bird community, and given that nutrient additions should most directly and strongly affect primary producers, we hypothesised that the responses of the wading bird community (if any) would be strongly related to their position in the food web. We expected that those birds that were most directly linked to fast turnover and/or lower trophic level resources (i.e. those that forage directly on plankton or invertebrates) should be affected more directly (sooner and with greater magnitude) by nutrient inputs than birds that are more removed from the invertebrate prey base (e.g. species that feed on fish or frogs). This hypothesis can also be viewed as an expression of the idea that populations of tertiary consumers should be buffered by the existence of dietary flexibility and greater quantities of stored nutrients (in biological tissue) at primary and secondary trophic levels. For example, a bird that feeds on a generalist fish species should be unaffected by changes in the fish's prey community, provided that the fish population still has access to a suitable volume of food. Obviously, many birds have the option to leave a particular system or forage more widely if food declines below acceptable levels.

We anticipated three possible outcomes of the analysis. The first was that the resource pulse might have no effect on wading birds over the time frame of data collection. This would imply that either there were no changes in the underlying prey base, or that some form of compensation (e.g. prey switching) occurred, or that system dynamics were slower than we could detect. Second, there might be an increase in wading bird numbers and/or species richness (keeping in mind that the system is open and appears to be dominated by movement rather than recruitment). This would imply an increase in prey populations. Third, there might be a decrease in wading bird numbers and/or species richness. This would imply a decrease in available prey, resulting from either a shift in the prey community to less edible species or a decrease in prey abundance. In addition, as argued above, responses were expected to occur differentially in bird populations that occupy different trophic levels. Each of these possibilities has different implications for our understanding of the effects of resource pulses on food webs in (and around) lake ecosystems. They would also suggest quite different levels of concern on the part of Barberspan management over the potential for further nutrient pulses in the study system.

Methods

Study site

Barberspan (26°33′S, 25°37′E; Fig. 1) is one of South Africa's 20 Ramsar-listed sites. According to the Ramsar Convention's web site (http://www.ramsar.org), it is one of the few permanent, natural water bodies in the highveld (i.e. the higher elevation, central region of South Africa). Barberspan is an alkaline, freshwater lake, surrounded by grassland and with rich plankton and fish communities. The fishery is not significantly managed and no additional stocking occurs. Barberspan is considered seasonally important for staging and breeding birds and locally and regionally migrant waterbirds (Milstein, 1975).

Barberspan was connected to the Harts River in 1913 and since then has contained water throughout the year, although considerable seasonal fluctuations in the water level occur. Its surface area varies between about 1000 and 1700 ha. It is adjacent to Leeuwpan, a smaller and more saline waterbody just to the north. Some water movement occurs from Barberspan to Leeuwpan.

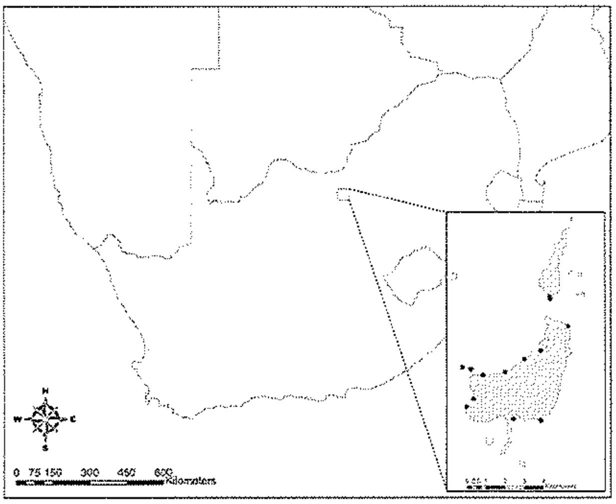

Fig. 1 Location of Barberspan in Southern Africa, indicated by the box, and sampling site locations, marked with a filled circle, on the shorelines of Barberspan (to the south) and Leeuwpan. Note that all sites were at the water's edge; wetland levels fluctuate seasonally and this 1 : 50 000-scale data layer does not encompass all of the shallower areas of the two dams.

Sampling design

We sampled 12 sites located around the shoreline of Barberspan and one site at Leeuwpan (Fig. 1). The same protocols were applied at all sites, every 2 months over the period March 2007–March 2010 (bird data) and January 2008–November 2009 (water quality data), respectively.

Water quality was measured at each bird counting site using an HI9828 multi-parameter probe (Hanna Instruments, Cape Town, South Africa). The same probe was used throughout the project and was recalibrated and checked according to the manufacturer's guidelines prior to each sampling event. It provided direct measures of dissolved oxygen, water temperature, pH, total dissolved solids and salinity.

Water was sampled using a protocol in which we started by taking a reading 1 m from the shoreline. The sampler then stepped forward 2 m and took another reading. This process was repeated until the person was either 21 m from the shoreline or at the maximum depth permitted by their waders. The probe was held just under the water surface for all readings. Water quality readings were averaged for each sampling occasion for the analysis. Samples were taken during the warmer periods of the day (i.e. between about 10 a.m. and 4 p.m.).

We counted birds every 2 months over a 3-year period at 13 fixed points using a standardised point count protocol. Each count consisted of 10 min of habituation time followed by a 30-min counting period, during which we counted all birds in front of us in a semi-circle with a radius of 150 m. The limits of each count were determined using a laser range-finder and obvious landmarks. During counts, birds were recorded as foraging, roosting or flying over, but we used only the foraging data for this analysis. During each sampling occasion (i.e. every 2 months), we counted each site in a random sequence four times, at different times of day (but always when visibility was sufficient for full identification), over a 5-day period. Each 5-day counting and water sampling period is termed a single 'mission'. Water quality measurements were taken during each mission so that we monitored the water conditions that the birds were experiencing. Additional details on the counting protocol and exact locations of all counts are published in Cumming *et al.* (2011).

For this analysis, we were mainly interested in birds that feed primarily on aquatic organisms (invertebrates, frogs and fish) and might reasonably be expected to be sensitive to changes in water quality via impacts on their prey base. The majority of these species are wading birds, which are also of high conservation concern in the management of the lake and an important component of its Ramsar status. We consequently limited our analysis to data from the families Charadriidae (plovers and lapwings), Ardeidae (herons and egrets), Recurvirostridae (stilts and avocets), Scolopacidae (sandpipers) and Phoenicopteridae (flamingos). Species are listed in Table 1.

Statistical analysis

Following basic descriptive analysis, we used CCA and partial Mantel Tests to test for a response by the bird community to water quality. We then explored the trajectories of changes in water quality and in the bird community by quantifying trends in time series of principal components.

The community analysis (CCA and Mantel tests) followed the recommendations of Legendre & Legendre (1998). We separated our data into three tables: a table of bird data, a table of environmental data and a table of covariates. All data were arranged in the same format, with each table having 156 rows that corresponded to each of our 13 sampling sites on each of the 12 missions for which we had matching bird and water quality data. Each cell in the bird table contained an average abundance for each species over four counts. The water quality data included average values per site per mission for each of dissolved oxygen (mg L^{-1}), pH (standard units), water temperature (°C), salinity (psu) and total dissolved solids (ppm). The covariates included month, year, X and Y coordinates (of counting and sampling points), distance to

Table 1 Common bird species in each different foraging group and accompanying Mantel test r (partial Spearman's coefficient, significance tested with 999 random permutations) and probability values for the correlation between wader community composition and water quality (dissolved oxygen, pH, salinity, total dissolved solids and temperature), corrected for spatiotemporal covariates (longitude and latitude of sampling sites, including nonlinear combinations; month, year and distance to river mouth). Note that lesser flamingo, included here with in-water invertebrate feeders, is a filter-feeding herbivore (greater flamingoes eat crustaceans). Further details in text.

	Where they feed		
What they eat	Dry land	Fringe	Water
Invertebrates	Blacksmith lapwing (*Vanellus armatus* Burchell) crowned lapwing (*Vanellus coronatus* Boddaert) cattle egret (*Bubulcus ibis* Linnaeus) Kittlitz's plover (*Charadrius pecuarius* Temminck) Mantel's $r = 0.01$ $P < 0.42$	Three-banded plover (*Charadrius tricollaris* Vieillot) common sandpiper (*Actitis hypoleucos* Linnaeus) curlew sandpiper (*Calidris ferruginea* Pontoppidan) common ringed plover (*Charadrius hiaticula* Linnaeus) little stint (*Calidris minuta* Leisler) Mantel's $r = -0.096$ $P < 0.98$	Black-winged stilt (*Himantopus himantopus* Linnaues) greater flamingo (*Phoenicopterus ruber* Linnaeus) lesser flamingo (*Phoenicopterus minor* Geoffroy) common greenshank (*Tringa nebularia* Gunnerus) ruff (*Philomachus pugnax* Linnaeus) pied avocet (*Recurvirostra avosetta* Linnaeus) marsh Sandpiper (*Tringa stagnatilis* Bechstein) African snipe (*Gallinago nigripennis* Bonaparte) wood sandpiper (*Tringa glareola* Linnaeus) Mantel's $r = 0.246$ $P < 0.001$
Vertebrates	Black-headed heron (*Ardea melanocephala* Anon) Mantel's $r = 0.002$ $P < 0.41$		Goliath heron (*Ardea goliath* Cretzchmar) grey heron (*Ardea cinerea* Linnaeus) little egret (*Egretta garzetta* Linnaeus) little bittern (*Ixobrychus minutus* Linnaeus) yellow-billed egret (*Egretta intermedia* Wagler) great egret (*Egretta alba* Linnaeus) squacco heron (*Ardeola ralloides* Scopoli) purple heron (*Ardea purpurea* Linnaeus) black heron (*Egretta ardesiaca* Wagler) Mantel's $r = 0.067$ $P < 0.1$

river mouth (the potential entry point of pollutants) and a set of nonlinear interaction terms of the X (easting) and Y (northing) coordinates, as recommended by Borcard, Legendre & Drapeau (1992). The nonlinear spatial terms included in the covariates matrix were X^2, Y^2, $X*Y$ and $(X^2 + Y^2)^{0.5}$. The results of ordination analyses are sensitive to the magnitudes of variables, so we standardised each column of data prior to the analysis by subtracting its mean and dividing by its standard deviation. Since Bray–Curtis distance cannot be calculated for negative distances, we added an arbitrary constant of 10 to each standardised value.

All analysis took place in the R statistical package (R Development Core Team, 2010), using routines from the 'VEGAN' library (Oksanen *et al.*, 2011). Each of the three matrices was converted to a dissimilarity matrix and we ran nonparametric Mantel tests (using Spearman's coefficient) between each matrix as well as a partial nonparametric Mantel test between the bird and water quality data, correcting for covariates. Partial Mantel tests were run initially on the full matrix of bird species ($n = 34$), and we then removed six rare species (i.e. those sighted on only one or two missions) from the analysis as well as comparing groups of birds individually using a simple, *a priori* foraging guild classification (as described in Table 1).

Canonical Correspondence Analysis was used to visualise the dominant axes along which individual samples were separated. By running CCA on the bird community data first and then using the 'envfit' option in the vegan library in R, we were able to visualise the bird community in relation to the dominant gradients of environmental variation. We used the 'varpart' command in the vegan library (i.e. sums of the proportional values of eigenvectors) to establish the total proportion of variance explained by our environmental and covariate matrices, respectively.

To better relate water quality changes to changes in the wading bird community, we calculated principal components for water quality and for the bird community independently of one another. For this calculation, we used data that were averaged by site to give a single number for each variable (i.e. either water quality variable or numbers of a given bird species) for each mission. We were then able to track the progress of the system across this multivariate 'state space', with the expectation that if declining water quality were pushing the bird community away from a locally stable state, component scores for the birds would be furthest from this state during the period when water quality was poorest. Given that we were monitoring a system that started off relatively unperturbed, went through a single, large perturbation, and returned to close to its starting state, we decided to use the first point of each time series as a reference point and to explore system movement in ordination space by taking the Euclidean distance of each data point (one point per mission for each data set) from its starting point at each time step.

Results

We first present individual analyses of water quality changes and the dynamics of the bird community and then focus on interactions.

Water quality changes

Dissolved oxygen levels did not differ between different sites within missions (two-way ANOVA, $F = 1.27, P < 0.24$), suggesting fairly high levels of mixing within the lake, although as discussed later, there was significant spatial variation in water quality between sites during some sampling periods. However, there was a significant change in dissolved oxygen through time (two-way ANOVA, $F = 37.65, P < 0.0001$), with a rapid decrease across all 13 sampling sites from early April 2008 through to late January 2009 (Fig. 2). Dissolved oxygen only recovered to pre-disturbance levels by July 2009, when the mean dissolved oxygen value was within the range of values obtained in January 2008.

Water quality recovery appeared to be complete by September 2009 but the relatively large range of dissolved oxygen values across sites in November 2009 (as indicated by the standard deviations displayed in Fig. 2a) suggested the addition of further nutrients following summer rainfall events, probably caused by overflow from upstream sewage holding ponds (Gouws *et al.*, 2010). This claim is supported by the very low dissolved oxygen values (0.09 and 0.49 mg L^{-1}) at the two sites closest to the river mouth (0.38 and 1.57 km away, respectively), through which sewage would enter the system; more remote sites had higher dissolved oxygen levels. With data for the two sites nearest the river mouth excluded, the mean dissolved oxygen value across all sites in

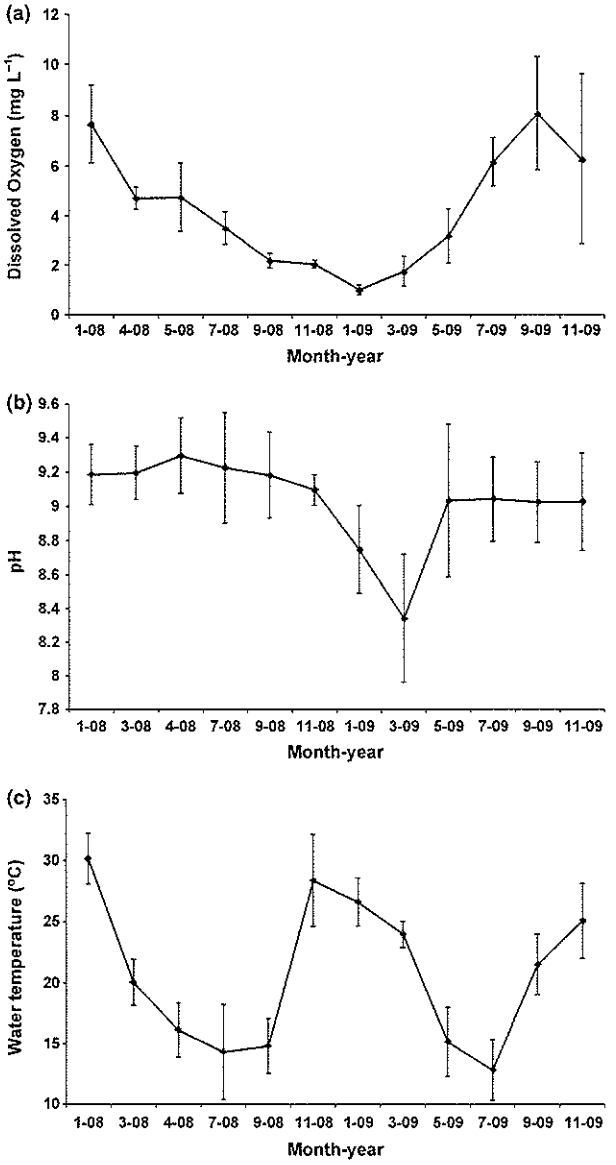

Fig. 2 Changes in different water quality variables over the 24-month water quality sampling period. The error bars indicate one standard deviation from the mean. Variables are (a) dissolved oxygen, in mg L^{-1}; (b) pH; and (c) water temperature, in °C.

November 2009 increased from 6.2 to 7.3 mg L^{-1} and the standard deviation decreased from 3.4 to 2.3.

The time series of pH values also showed significant variation (one-way ANOVA, $F = 11.85$, $P < 0.0001$), with normally alkaline Barberspan water becoming significantly less alkaline in January and March 2009 (Fig. 2b). There was no significant variation in salinity levels (d.f. = 11, $F = 0.47$, $P < 0.92$) or total dissolved solids ($F = 0.43$, $P < 0.93$) over our sampling period. By contrast, and as would be expected, water temperature varied significantly through the year ($F = 69.5$, $P < 0.0001$) with as much as a 12 °C difference between summer and winter months (Fig. 2c).

Bird data

We first tested for differences in wading bird numbers between counting sites within each of the 12 missions, using a two-way ANOVA. The mean probability value for all between-site ANOVAs ($n = 34$ species, 12 missions) was $P < 0.11$, with a standard deviation of 0.13, indicating that some sites differed significantly during the sampling period. A two-way ANOVA of the mean counts for all missions (including those when water quality was not measured) suggested that significant differences also occurred within individual sites between missions ($F = 1.78$, $P < 0.02$, $n = 37$ bird species and 19 missions). For the subset of 12 counts for which we also had water quality data, greater differences were evident (two-way ANOVA $F = 2.52$, $P < 0.005$). These results indicated that significant changes occurred in both the abiotic environment and the bird community during our sampling period.

Bird–environment interactions

A partial Mantel test comparing bird and environmental data by site and by mission, correcting for spatial and temporal covariates, yielded a significant correlation (Mantel's $r = 0.13$, $P < 0.016$, 999 permutations, $n = 34$ bird species). This indicated that independently of the influence of site (space) and mission (time) on both the bird community and the environmental variables, a significant relationship existed between water quality and the bird community. With six rare species excluded from the analysis (as discussed in the methods section) this relationship strengthened (Mantel's $r = 0.16$, $P < 0.001$, 999 permutations).

The Mantel's r values for pairwise correlations between the three matrices (using 28 bird species) were all significant. For waders and environmental data, $r = 0.24$, $P < 0.001$; for waders and covariates, $r = 0.33$, $P < 0.001$; and for environmental data and covariates, $r = 0.30$, $P < 0.001$. These statistics suggest a strong influence of the spatiotemporal covariates (i.e. seasonal drivers) on both water quality and the bird community. However, the partial Mantel test indicated that independently of seasonal dynamics, there was still a significant influence of water quality on the bird community during the study period.

Canonical Correspondence Analysis suggested that the dominant environmental influences on the bird community were pH, dissolved solids and salinity (first component), and water temperature (second component, primarily reflecting seasonality). These variables explained a significant proportion of variance in the CCA axes ($P < 0.001$). Dissolved oxygen, by contrast, did not emerge as a significant influence in the analysis ($r = 0.13$, $P < 0.3$). Water quality, site and mission together explained just over 20% of the variance in the wading bird community.

Mapping the bird community and the environmental data together in a single ordination (Fig. 3) suggested that different foraging guilds of wading birds (Table 1) were separating along different environmental gradients. Qualitatively, greater and lesser flamingos appeared to be strongly influenced by salinity (as expected, given their preference for saline Leeuwpan); pH was the dominant influence on sandpipers and other probe-feeders; the small shoreline foragers, such as Kittlitz's plover, little stint and common ringed plover, fell along a water temperature axis (most likely a proxy for seasonality); and the larger herons and egrets, which eat mainly frogs and fish, were less impacted by water quality.

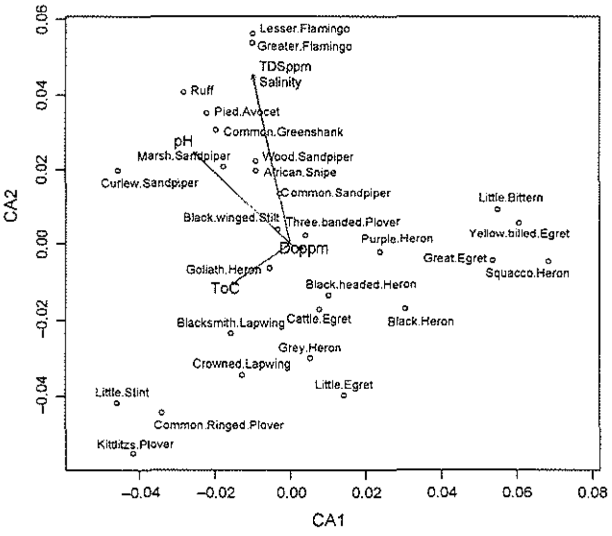

Fig. 3 Canonical correspondence ordination of data for the wading bird community, with the axes (dominant gradients) of environmental variation identified by the analysis overlaid.

To provide a more rigorous test of the hypothesis that birds were responding by foraging guild, we ran partial Mantel tests by foraging guild (i.e. correlating wading birds and environmental variables, with covariates corrected for). The results (Table 1) indicated that the main source of the observed impact of water quality on the wading bird community was the guild of birds that forage on invertebrates in slightly deeper water, away from the immediate fringe of the pan [$r = 0.25$, $P < 0.001$; results for other groups were statistically insignificant (Table 1)].

Trajectory analysis

Plots of the first two principal components from the environmental data and the wading bird data, respectively (Figs 4a,b), show that both data sets returned to their starting point towards the end of the sampling period. Both are furthest from the starting point during the period when water quality was at its lowest, from July 2008 to January 2009. The first two components for the bird community data explained 25 and 19% of variation, respectively. For water quality data, the first two components explained 33 and 23% of variation, respectively. The Euclidean distance of the principal component scores for each mission from the centre of the ordination offers an indication of the proximity of the system to a central attractor. These distances were significantly correlated ($r = 0.61$, $P < 0.03$, $n = 12$) for the bird community and water quality data at a lag of one sampling period (2 months) but not when unlagged or at a lag of 3 months, indicating that the abiotic and biotic components of the system are coupled (Fig. 4c). For the water quality data, the return time of the system was *c.* 16 months; for the bird community, the return time was *c.* 8 months, or half that of water quality (Fig. 4c).

Discussion

Our analysis identified several clear and ecologically important patterns. Following nutrient addition, there was a significant decline in water quality at Barberspan, particularly during the second half of 2008, with pH decreasing and dissolved oxygen levels dropping to below 2 mg L^{-1}. It is important to note that (i) this was a large and ecologically meaningful decline and (ii) neither this decline nor the related changes in the bird community were at all synchronised with typical southern hemisphere lake dynamics or southern hemisphere seasons (and hence seasonal events such as the arrival of Palearctic-breeding migrants or flooding in the Harts River had no impact on our findings). In fact, keeping in mind that Barberspan is in a relatively arid, summer

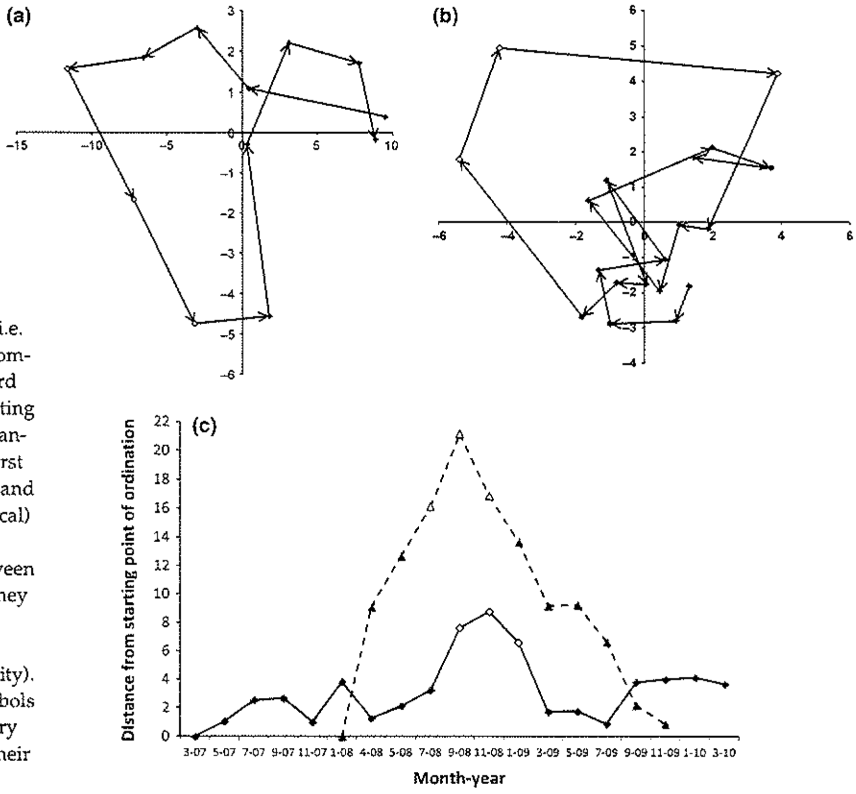

Fig. 4 Sequential values by sampling mission (i.e. every two months) for the first two principal components of (a) all environmental data; (b) all bird count data; and (c) Euclidean distance from starting point of bird (diamonds) and water quality (triangles) data, respectively. For both a and b, the first component is plotted on the x (horizontal) axis and the second principal component on the y (vertical) axis. The arrows show the direction of system movement in time. The lagged correlation between the plots in a and b is visually obvious when they are viewed as (c) Euclidean distances from the starting point (triangles and dashed line, water quality; diamonds and solid line, bird community). In all figures, points represented by hollow symbols mark the period from September 2008 to January 2009 when dissolved oxygen and pH were at their lowest.

rainfall region, rainfall in November 2008 should have oxygenated rather than deoxygenated the water column.

As a reference point, one of the best-studied lakes in southern Africa is Lake Chivero, a 2600 ha impoundment that provides drinking water for Harare, the capital of Zimbabwe (*c.* 1000 km from Barberspan). Magadza (2003) blamed increasing sewage inputs to the lake for the decline of dissolved oxygen in Lake Chivero to levels comparable to those experienced by Barberspan during this study. This same decline was evident in a wide range of other water quality parameters at Lake Chivero and periodic fish kills continue to occur (Magadza, 2003). The data from Lake Chivero from earlier, pre-eutrophication periods also indicate strongly that the levels of dissolved oxygen encountered in Barberspan are very far from 'natural' for southern African lakes (see Gouws *et al.*, 2010, for further information on pollution at Barberspan).

Mortality rates of freshwater fish start to increase once oxygen concentrations fall below 5 mg L^{-1}, with concentrations below 3.0–3.5 mg L^{-1} resulting in significant mortality within 24–48 h at water temperatures in the range 11–19 °C (Landman, Heuvel & Ling, 2005; Moore, 1942). Although no data were available describing the benthic invertebrate fauna of Barberspan, small freshwater shrimps *Paratya curvirostris* in New Zealand experience 50% mortality within 48 h at dissolved oxygen concentrations of ca 1 mg L^{-1} at 17 °C (Landman *et al.*, 2005) and low oxygen levels negatively impact benthic populations of chironomids and oligochaetes (Doke *et al.*, 1995).

Water quality at Barberspan appears to have recovered to pre-perturbation levels by September 2009, although high variance in dissolved oxygen concentrations in November 2009 suggests that a further input of sewage may have commenced towards the end of our study period. The changes in water quality were reflected numerically in the community of wading birds, particularly in the medium-sized waders and most notably the Scolopacidae. The most affected foraging guild was that of planktivorous and invertebrate-eating birds that forage in the sub-littoral zone, supporting the hypothesis that birds that feed on high-turnover populations and/or at lower trophic levels will be more directly affected by resource pulses.

The wading bird community took 2–3 months to respond to the resource pulse, as indicated by the lagged correlation values, but recovered rapidly (within *c.* 2 months) once water quality started to improve (Fig. 4b). This in turn suggests that invertebrate responses to lowered water quality only occurred at or beyond a threshold level, with invertebrate prey availability for wading birds being largely unaffected during the early and late stages of the resource pulse; or alternatively (and less probably, given the time periods involved), that birds took some time to detect changes in prey abundance. The higher turnover of the invertebrate prey base means that it should recover faster than that of frogs and fish. We thus propose that two different mechanisms may be pulling in different directions to determine the sensitivity of secondary and tertiary consumers to changes in their prey. First, for consumers that forage lower in the food web, less buffering by generalists occurs in the intervening trophic levels (serving to increase sensitivity). Second, the dynamics of populations lower in the food web tend to be faster, making recovery faster (and serving to decrease sensitivity).

There has been little research on the impacts of water quality on communities of freshwater wading birds in southern Africa (but see Ashkenazi, 2001; Kalejta-Summers, Mccarthy & Underhill, 2001; Velasquez, 1992). Given that the smaller wading birds that appear to have driven the trend in our data can switch readily between different invertebrate prey items (Hockey & Turpie, 1999) and that many oligochaetes and dipteran larvae are both eaten by birds and tolerant of poor water quality (Rosenburg & Resh, 1992), we were surprised to find such a strong response by the bird community. Reductions in pH can negatively impact prey items with calcareous shells (e.g. Malley, 1980), but Barberspan is unusually alkaline under normal conditions and remained alkaline during this study despite a two-unit decline in pH. Although we have documented correlation rather than causation, and although our observations are based on a single 'natural experiment' rather than a repeated experimental design, there are no other reasonable hypotheses that explain the synchronous changes in abiotic and biotic systems. The shift away from starting conditions does not occur at the same time as other major influences on either the birds or the water, such as the annual arrival of Palearctic-breeding migrants or the onset of the rainy season (Hockey, 2000; Hockey, Dean & Ryan, 2005), or other activities that might possibly have impacted water quality (e.g. sport fishing and the one-off removal of reed beds for boating and quelea *Quelea quelea* control). We thus conclude, subject to the limitations of our data set and analytical approach, that the main mechanism explaining the observed patterns was a reduction in prey abundance or availability, following reduced water quality, during the period when dissolved oxygen and pH were at their lowest extremes. Regardless of the exact mechanism, our results indicate that (i) wading birds that forage directly on invertebrates can be negatively affected by resource pulses and (ii) different guilds within the wading bird community respond differently to resource pulses.

Lastly, given that conservation of wading birds (and other waterbirds) is one of the primary motivations for protecting Barberspan and that habitat provision for both migrant and resident waders constitutes one of its main claims to Ramsar status (Milstein, 1975), it appears that urgent action to limit lake eutrophication is needed before Barberspan reaches a tipping point and becomes irreversibly eutrophic.

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ORIGINAL ARTICLE

Towards a unification of movement ecology and biogeography: conceptual framework and a case study on Afrotropical ducks

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ABSTRACT

Aim We present elements of a new conceptual framework for the unification of biogeography and movement ecology, and demonstrate the value of the new framework using a case study of two species of Afrotropical duck (Anatidae) across a latitudinal gradient.

Location Southern Africa.

Methods The first part of the paper rests on logic and philosophy. For the second (case study) section, we used data from up to 3 years of satellite telemetry for 26 individuals of two species of Afrotropical duck, the Egyptian goose (*Alopochen aegyptiaca*) and red-billed teal (*Anas erythrorhyncha*), from three different populations with moulting sites spread across 17 degrees of latitude. We compared quantitative measures of movement patterns using principal components analysis and boxplots.

Results We argue that unpacking the concept of dispersal into the fundamental elements of movement (internal drivers, external drivers, navigation capacity, and motion capacity) provides a more solid basis for contrasting competing hypotheses in biogeographical studies. We found, surprisingly, that red-billed teal, a 'highly nomadic' species, moved with a relatively high degree of consistency at each of our three study latitudes, while Egyptian geese, a 'resident' species, showed latitude-dependent variation in their movements. However, much of the latitude-related variation for Egyptian geese was driven by their annual moult migrations, rather than directly by fluctuations in resource availability. Internal factors appear to dominate movements and probably determine the species ranges of both of our study species.

Main conclusions The integration of biogeography and movement ecology, through a more sophisticated view of mechanisms formerly lumped together under 'dispersal', offers a fertile area for further research. The biogeography of Afrotropical ducks appears to be strongly influenced by internal factors. Biogeographical patterns in this taxon may thus be best understood (and modelled) as a long-term response to environmental stochasticity, rather than as a deliberate selection of optimal habitat. Latitudinal comparisons emerge as a valuable way of gaining insights into the drivers of movement for widespread species.

Keywords

Africa, *Alopochen aegyptiaca*, *Anas erythrorhyncha*, Anatidae, biogeography, dispersal, movement, niche, species range.

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INTRODUCTION

The field of biogeography has its origins in observations of the spatial patterns of species occurrences. Classical biogeographical writings (e.g. von Humboldt & Bonpland, 1807 (reprint 2010); Darwin, 1859) dwelled on the influences of climate and landform on the distributions of plant and animal communities. Early biogeographical theories, such as vicariance biogeography, recognized the need to incorporate movement, or lack of movement, to explain anomalies in species ranges such as disjunct distributions (e.g. Bermingham *et al.*, 1992). However, movement was considered in a relatively coarse-grained fashion, focusing on broad-scale barriers to movements such as oceans, deserts and mountain ranges.

The importance of movement as a determinant of species ranges achieved greater prominence with the formulation of the theory of island biogeography (MacArthur & Wilson, 1967; Lomolino & Weiser, 2001), which highlighted the explanatory power of differential immigration into ecological communities as a function of distance from source pools of potential community members. It is now widely recognized that spatial patterns in species occurrences result from the interplay of spatio-temporal variation in the environment and the responses of organisms to this variation and to one another (Franklin & Miller, 2010). Movement offers (among other things) a way of escaping unsuitable environments, improving mating chances, avoiding competition, and exploiting resource pulses (Nathan *et al.*, 2008). Broad-scale environmental influences on movement, and the consequences of movement for biogeographical patterns and processes, are, however, still quite poorly understood (Holyoak *et al.*, 2008). Biogeographers continue to focus primarily on static records of species occurrences, with the vast majority of species occurrence models being based on assumptions of environmental control of species ranges (e.g. Cumming, 2000; Algar *et al.*, 2009; Franklin & Miller, 2010) rather than on models that explicitly consider species movements and seasonal variations in home range and life history requirements. There thus appears to be a clear need for better integration between traditional biogeographical studies, on the one hand, and movement ecology, on the other (see also Guisan *et al.*, 2006).

Achieving a better level of integration between biogeography and movement ecology will require a considerable amount of additional research, and we do not claim to have all the answers for how this should be achieved. Here we focus on two related goals: (1) to define the problem, and in so doing to offer a first set of suggestions for how movement and biogeography can be better integrated; and (2) to present an exploratory case study, focusing on the biogeography and movement ecology of two African ducks.

Integrating biogeography and movement ecology

Species ranges can be viewed as the outcome of an organism's evolutionary history and phylogeography, fundamental and realized niches, environmental influences, and movements (e.g. Chase & Leibold, 2003; Broennimann *et al.*, 2006). It follows that some biogeographical patterns may be best explained not simply by the distances between locations and/or so-called 'barriers to dispersal', but by more complex mechanisms that derive from the other aspects of movement. We would expect this to be particularly true for animals that (1) are relatively mobile and (2) have stronger cognitive capabilities for information processing. Examples of taxa for which movement seems most likely to be a key biogeographical influence include birds, bats and marine mammals.

Integrating biogeography with current trends in movement ecology requires an unpacking of the assumptions that are contained in the idea that distance limits dispersal. Movement by an individual organism is itself an outcome of a number of related influences, both internal and external. Nathan *et al.* (2008) argued that animal movement, in the form of a movement path, should be understood as a consequence of (and subsequent influence on) the interaction of four factors: internal state, navigation capacity, motion capacity, and external factors. Understanding the relevance of each of these factors to a given organism's movement path, at multiple scales, provides the basis for understanding its movement ecology. Although it might be argued that many of these elements are contained in the idea of a fundamental niche, to do so trivializes the important role that acquired or learned behaviours can play in animal movements. For example, reintroduction programs have demonstrated convincingly that young sandhill cranes (*Grus canadensis*) have to be taught to follow ancestral migration routes (Ellis *et al.*, 2003). While an animal's basic biology equips it with a certain body plan and capacity to move, niche concepts alone do not explain some of the glaringly obvious differences in range sizes between apparently similar species, nor those between different populations of the same species that have distinct ranges (e.g. barnacle goose, *Branta leucopsis*, or pink-footed goose, *Anser brachyrhynchus*) (Scott & Rose, 1996).

The process of biogeographical analysis typically takes place at one of three levels of explanation: evolutionary, ecological and individual. Explanations from different levels of explanation are complementary rather than contradictory. For example, the species range of a Palaearctic bird that migrates annually from Europe to Africa can be explained simultaneously (and equally 'correctly') as a consequence of selective pressures exerted by a changing climate and environment over millennia, as a seasonal response to declining food availability in autumn, and as a physiological response to reduction in day length. The potential for multiple correct explanations from different levels must be kept in mind when considering explanations for biogeographical patterns that are based on empirical analyses of movement, because movement analyses generally focus on an ecological level of explanation; the presence of complementary (and equally correct) explanations at evolutionary and individual levels does not, however, preclude additional ecological explanations.

The biogeographical analysis of ecological influences can be described as a process of working down a hierarchical decision tree in which competing hypotheses about the limits to a species' range are contrasted (Cumming, 2002, 2007; Fitzpatrick *et al.*, 2007; Freckleton & Jetz, 2009). The hypothesis that has the best explanatory power is the one that most closely captures the current species range limit (keeping in mind that the best hypothesis may vary for different range boundaries, for example if the northern limit is determined by food availability and the southern limit by the extent of the continental land mass). Ecological explanations of current biogeographical patterns consist of tests of hypotheses from three main categories – environmental, movement-related or historical (including long-term influences on genes; Seaman *et al.*, 1999) – that might explain why a species does *not* have a larger species range (Fig. 1; Parmesan *et al.*, 2005; Guisan *et al.*, 2006; Cumming, 2007).

Tests of the movement-related hypotheses in Fig. 1 clearly go further than simply looking at dispersal distance and the proximity of suitable patches of habitat. Rigorous tests of these hypotheses will require a combination of empirical movement data, species occurrence modelling, statistical phylogeographical studies of genetic data (Richards *et al.*, 2007) and dynamic movement models. One of the most obvious challenges in this context is to incorporate studies of individual and population-level movements in the quantitative estimation of broad-scale patterns of species ranges and in predictions of changes in species ranges in response to environmental drivers such as deforestation and climate change.

Hypotheses about the impacts of movement on species occurrences can be tested experimentally via habitat manipulation, relocation, or laboratory-based analyses of captive organisms from different locations; or pseudo-experimentally by monitoring range extensions of introduced species (e.g. Lensink, 1998). Experimental studies, however, have the dual disadvantages that: (1) they interfere with natural movement patterns; and (2) they are not practical for many far-ranging animals, such as nomadic or migratory birds.

An obvious but little-used alternative for field-based research on the influence of movement in biogeography is to contrast movement data from populations of the same species that occur in different locations along one or more broad-scale environmental gradients (e.g. see Hosseini *et al.*, 2004; Parmesan *et al.*, 2005). In this way, internal and historical factors (e.g. navigation capacity, movement capacity, evolutionary history) can be held relatively constant while the external environment is altered. Differences and similarities in movement patterns along broad-scale environmental gradients can be used to draw strong inferences and guide hypothesis testing about species range limits (Table 1).

A case study: latitudinal movements and biogeography of two species of African duck

Biogeographical background

As a test case we next consider the biogeography of two species of Afrotropical duck: the Egyptian goose, *Alopochen aegyptiaca* (Linnaeus) (noting that Egyptian geese are shelducks, subfamily Tadorninae, rather than true geese) and red-billed teal, *Anas erythrorhyncha* Gmelin.

Considering these species in relation to the hypotheses in Fig. 1, a preliminary inspection of what is known about their species ranges (Fig. 2) and biology suggests that a case can be made for either environmental or movement limitation for each of them. Red-billed teal occur extensively throughout most of southern and East Africa but are notably absent from Central and West Africa, while Egyptian geese occur across

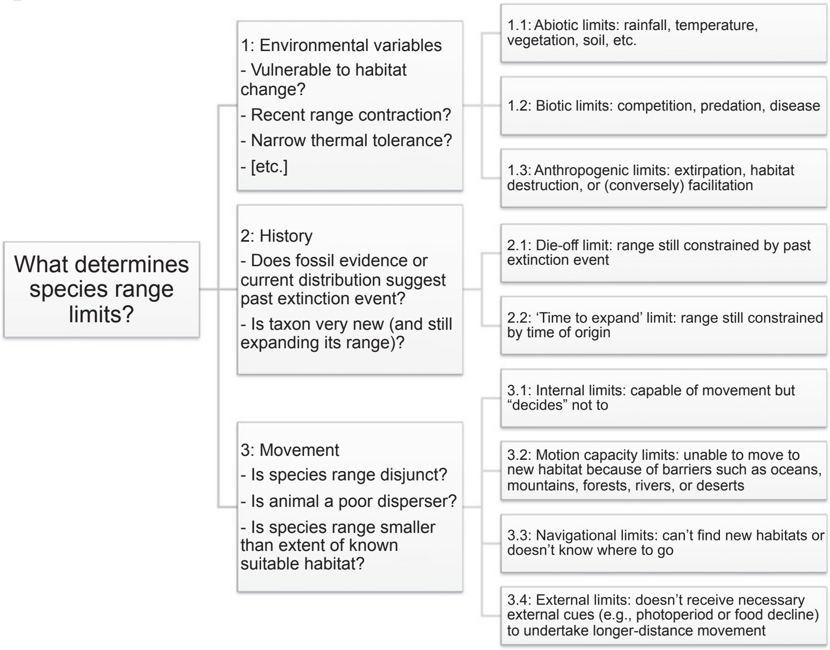

Figure 1 Summary of the three major classes of competing hypothesis that might explain species occurrences. Our central argument in this paper is that the study of biogeography would benefit from a more rigorous and detailed unpacking of the third box, movement, in accordance with recent developments in movement ecology.

Table 1 Summary of differences in movement patterns that might be observed in a movement-limited population when comparing observations from different populations along a broad-scale gradient. These hypotheses must be contrasted with competing hypotheses, as summarized in Fig. 1; they all assume that responses to environmental variation, for example, are not limiting, although it would obviously be possible for biogeographical patterns to be determined by several different influences that have different effect sizes at different times of year or under different conditions.

Primary movement-related factor that limits range expansion	Expected movement pattern along a broad-scale environmental gradient	Empirical data to focus on when testing a hypothesis	Competing hypotheses (at the same level of explanation) for the same pattern
Internal factors	No consistent differences in movement patterns along a gradient; different individuals may behave in quite different ways within the same population	Daily distance travelled shows no relationship to resource availability or season; longer movements driven primarily by life history needs, such as mating, nesting, moulting or dispersal	High influence of biotic environment (e.g. competition for nest sites)
Navigation capacity	Limited range of movements (e.g. repeated use of a small number of routes) despite much wider habitat availability	Individual movements expected to be consistent, even if population-level trends are not. Relocation experiments can test ability to return 'home'	Awareness of broader landscape pattern only evident during periods of stress (e.g. drought)
Movement capacity	Matches classical assumptions: tendency to remain within a particular habitat type and/or to not cross obvious barriers and boundaries	Disjunct ranges with no dispersal between populations, clear relationships to obvious barriers, and other well-documented patterns. Most relevant to smaller and less mobile organisms; amenable to relocation experiments	Habitat specialization versus movement limitation can be difficult to distinguish. Other movement-related factors (e.g. navigation capacity) may still be important
External factors	Consistent differences in movement patterns (e.g. distance and seasonal direction) expected between populations, correlating with the relevant gradient	External factors are expected to drive movement in cases of environmental limitation. For external factors to *limit* movement to new habitat requires a compartmentalized system in which local resources fluctuate but are not limiting and there is no regional sampling of habitat by the organisms. Empirical focus needs to be on contrasting scales of resource availability in relation to movement. External social factors (e.g. need to remain with a flock, herd, or school for predator defence) may also limit movement	Can be difficult to distinguish from environmental limitation. Key questions include: (1) whether resources are limiting at any time of year, under normal conditions; and (2) whether social demands constrain broader-scale habitat searches/surveys

most of sub-Saharan Africa (in both cases with the exception of heavily forested areas) (Brown *et al.*, 1982; Scott & Rose, 1996). Both species are capable of long-distance movements, with maximum displacement distances recorded from ring recoveries being > 2000 km for red-billed teal and > 1000 km for Egyptian goose (Underhill *et al.*, 1999).

The smaller range of the red-billed teal may imply environmental limitation. However, there are several Palaearctic dabbling ducks in the genus *Anas* (e.g. *A. acuta*, *A. querquedula*) that have similar basic biology and habitat requirements to the red-billed teal, share habitat with red-billed teal in East Africa during the boreal winter, and are found in large numbers in southern Europe and West Africa during the boreal winter (Scott & Rose, 1996). There is no obvious evidence that habitat specialization, resource limitation, or physiological intolerance to colder conditions would limit the ability of red-billed teal to survive and breed either in southern Europe or in West Africa (e.g. see Petrie & Rogers, 1997; Underhill *et al.*, 1999; Hockey *et al.*, 2005). Interestingly, species ranges that are limited to southern and eastern Africa seem to be typical of all other Afrotropical *Anas* species (including, for example, *A. capensis*, *A. undulata*, *A. sparsa*, *A. hottentota* and *A. smithii*), while the ranges of most other Afrotropical ducks [e.g. the whistling ducks (Dendrocygninae) and comb duck, *Sarkidiornis melanotos*] extend into West Africa (Brown *et al.*, 1982; Scott & Rose, 1996).

Red-billed teal do not occur in the wild in Europe, but resident and apparently self-maintaining introduced populations of Egyptian geese occur in the north of Europe, including breeding pairs in Britain, the Netherlands, Belgium and Germany (e.g. see Lensink, 1998). In both southern Africa and Europe, Egyptian geese flourish in close proximity to human agricultural activities, in parks and on golf courses, and in urban environments with fields and lawns. The European

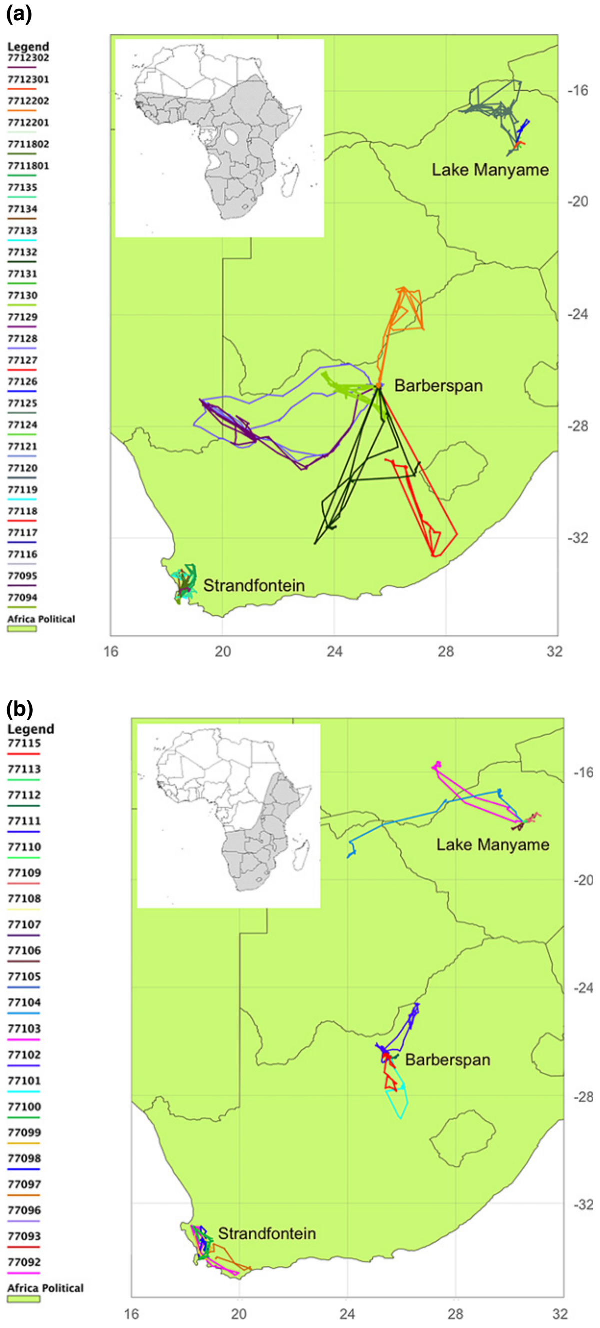

Figure 2 Overview of movement paths for all three southern African sites for (a) Egyptian goose (*Alopochen aegyptiaca*, *n* = 16) and (b) red-billed teal (*Anas erythrorhyncha*, *n* = 10). The insets show the African ranges of each species, redrawn from Brown *et al.* (1982).

populations of Egyptian geese provide strong evidence that the range expansion of African populations has not previously been limited by cold tolerance or food availability. Food availability prior to the expansion of human agricultural activities may of course have been limiting, implying that Egyptian geese may not have had sufficient time to colonize Europe naturally, but their rapid population expansion in southern Africa over the last 50 years does not support this argument.

Comparisons to congeners do not support a classical 'movement barrier' hypothesis. Ducks are strong fliers, and a large number of Palaearctic ducks overwinter in West and East Africa. Satellite tracking of various species of ducks closely related to Egyptian geese or red-billed teal demonstrates their ability to perform long-distance movements in a matter of days, including the direct crossing of large natural barriers such as the Sahara Desert or the Himalayan mountain chain (Gaidet *et al.*, 2010; Takekawa *et al.*, 2010). In terms of energetic expenditure and dispersal capacity, movement from the Sahel to the Mediterranean area should thus be an easy option for both species.

Working our way through the hypotheses outlined in Fig. 1, this discussion leaves us with the possibilities that range expansion in both Egyptian geese and red-billed teal has been prevented by: (1) some unmeasured environmental variable, such as predation, competition, or breeding habitat requirements; or (2) some element of their movement behaviour, including three of the four hypotheses summarized in Table 1 (i.e. excluding movement limitation). To test the second group of hypotheses we contrasted different movement-related hypotheses using latitudinal data from satellite-tagged birds from three locations in southern Africa, as explained below.

MATERIALS AND METHODS

We tagged birds with satellite GPS platform transmitter terminals (PTTs; Microwave Telemetry Inc., Columbia, MD, USA) at three latitudes in areas located along a north–south gradient running from Lake Manyame in Zimbabwe (17°49′ S, 30°36′ E, near Harare) through Barberspan (26°33′ S, 25°37′ E, North West Province of South Africa) to Strandfontein (34°05′ S, 18°32′ E), on the edge of False Bay, near Cape Town (Fig. 2). The tagging locations span 17 degrees of latitude (approximately 17° to 34° S), and the ends of the transect are situated *c.* 2200 km apart. Lake Manyame falls in a typical summer rainfall region; Barberspan receives variable but dominantly summer rainfall; and Strandfontein, just outside Cape Town, falls in South Africa's winter rainfall region.

Intuitively, one would expect the movement patterns of wetland-dependent species such as Egyptian geese and red-billed teal to relate closely to wetland area and spatial distribution; and the three study sites are in different biomes, different climate zones, and different agricultural regions. However, all of the birds were tagged in permanent wetlands and in areas in which wetland habitat is readily available. There is no evidence that either wetland or agricultural habitat (in the case of Egyptian geese) was in any way a limiting resource for any of the birds in this study. The same point has been made for other Southern Hemisphere species in relatively arid environments, such as the grey teal, *Anas gracilis*, in Australia (Roshier *et al.*, 2006, 2008).

Birds were captured at each of our three study sites using walk-in traps, mist nets and a cannon net. We tagged individual birds from the various traps and typically spread captures over several days to ensure that two birds from the same flock were not tagged. We deliberately selected known wing feather moulting sites for the study and tried where possible to place transmitters on adult birds that had recently moulted but had not completely regrown their wing feathers. We did this for three main reasons: (1) so that we would know definitively that the bird was tagged at a moulting site; (2) because birds that moult in a given location are more likely to be part of a local population, and hence to behave typically for that region, rather then being transient, lost, or vagrant individuals; and (3) because tagging recently moulted birds extends the time until the potential regrowth of trimmed back feathers over the solar panel of the PTT, as these birds do not moult their back feathers until the following year.

Transmitters were attached to birds using a backpack harness made out of teflon ribbon (Cumming & Ndlovu, 2011). We used 22-g transmitters for red-billed teal (mean adult weight of tracked birds, 642.24 g; standard deviation, 97.33 g) and 30-g transmitters for Egyptian geese (mean adult weight of tracked birds, 2299 g; standard deviation, 432.46 g). Transmitters were set to capture GPS location data every 2 h (30-g PTTs) or every 4 h (22-g PTTs). The 30-g transmitters were set to transmit data to the Argos satellite every 3 days, while the 22-g transmitters were queried every 5 days.

During the period of this study, and as part of a larger project (see Cumming *et al.*, 2011), we also captured and ringed over 730 Egyptian geese and 760 red-billed teal from the same three study sites. All unmarked captured birds were ringed with metal rings, and 710 Egyptian geese from Strandfontein and Barberspan were colour-ringed with Dacron rings that were colour-coded by site. Scans for colour rings formed part of bird counts that were undertaken every 2 months for a 3-year period (2007–2010) at both Strandfontein and Barberspan and have so far yielded a total of 81 returns. We thus had a strong basis from which to detect movement between sites, if it were occurring.

Both study species typically undergo flightless (synchronous wing feather) moult soon after breeding, meaning that movements during the post-moult period are representative of more general movement patterns in response to environmental variation, but not of breeding- and moult-related movements. Breeding takes about 14 weeks for Egyptian geese (4 weeks of incubation plus 10 weeks until the ducklings can fly), and moult takes over a month (Milstein, 1993). Timelines are slightly shorter for the smaller red-billed teal (Hockey *et al.*, 2005). Assuming that birds do not moult immediately on completion of breeding, any data set longer than about 7 months should thus include life history-related movements in addition to non-breeding foraging behaviour. We excluded data from birds that were tracked for less than 231 days (i.e. just over 7½ months). This threshold gave us effective sample sizes of $n = 16$ birds for Egyptian geese and $n = 10$ birds for red-billed teal.

Data processing and all analyses were undertaken in Matlab 2010b (MathWorks, Natick, MA, USA), using either custom-written routines or functions from Matlab's statistical toolbox. We screened each data set visually for outliers, but the GPS data for individual birds appeared to be of very high quality and there were no obvious outliers in any data set. The data are high resolution and broad extent (i.e. accuracy *c.* 10–20 m and extent up to 1500 km), and the study species are highly mobile, making smaller errors in location (e.g. at the scale of 50–100 m) both difficult to detect and irrelevant to the study's conclusions.

We quantified a range of descriptors of bird movement paths (Table 2). These measures were intended to capture a wide range of elements of the spatial and temporal use of habitat by individuals, as well as potential biases and sampling effects. We divided the variables into two sets (Table 2): (1) a set of variables that described individual movements ('movement variables'); and (2) a set of variables that were potentially confounding factors ('confounding factors'). The actual values for all variables are given in Appendix S1 in the Supporting Information.

Despite the relatively large sample size for a typical telemetry-based project, our total number of tracked birds (26 individuals of two species spread across three sites) was slightly too small to support a full-scale multivariate analysis with corrections for the main confounding factors. We thus used principal components analysis (PCA) of the confounding variables and the movement variables (independently) to reduce the dimensionality of each of these data sets (Legendre & Legendre, 1998). Data were standardized by column prior to PCA by subtracting the mean and dividing by the standard deviation. We then used boxplots of the first component to explore differences between sites and species.

RESULTS

Although movements between the tagging sites have been documented for both species from a few ringing recoveries, and some birds from Barberspan appear to move to the Cape seasonally (Oatley & Prys-Jones, 1986; Milstein, 1993), overlap between the different populations appears to be extremely rare at our tagging sites. We did not observe any overlap of tracked, metal-ringed or colour-ringed birds from different populations during the course of the study (Fig. 3), despite intensive captures at each site over a 2-year period and counts or an additional year following the cessation of captures. Populations at each of the three sites can thus be considered independent from each other.

There was considerable variation in the movement patterns of individual birds both within and between sites (Fig. 3 and Table 3). Egyptian geese from Barberspan tended to leave the site after moulting, while those from Strandfontein tended to stay within the winter rainfall region, and those from Manyame moved towards the Zambezi and Linyati Valleys to the north and west, and back (the Zambezi forms the northern border of Zimbabwe; the Linyati is in north-eastern Botswana). Egyptian geese from Lake Manyame on average moved

Table 2 Variables quantified in the analysis for each of the two species of Afrotropical duck. Type 'C' indicates variables that were treated as confounding factors; 'M' indicates movement variables.

Variable	Type	Units	Estimation method	Motivation
nrecords	C	N	Number of samples (re-sightings)	Influences data amount and quality
Transmitter days	C	days	Total number of days for which transmitter was active (last date – first date of transmission)	Influences data amount and quality. Similar to nrecords under ideal conditions, but different in some cases
total km	C	km	Sum of all individual distances between successive points (deg2 km)	Quantifies total distance travelled
startdate	C	date	Day of first record (excluding testing data) converted to Matlab serial day format (i.e. number of days since a hypothetical date of 1st January 0000)	For data sets less than a year in duration, the period that is monitored might influence observed movements
maximum daily rate	M	km/day	Calculate movement rate between each successive pair of GPS coordinates; divide by time between readings; find maximum value for data set.	Quantifies magnitude of occasional long-distance movements.
Hmean dailyrate	M	km/day	Harmonic mean of all daily movement rates (move ment rates calculated as above)	Mean movement rate; harmonic mean more appropriate here than arithmetic mean
kilometres per day	M	km	Sum of all distances between pairs of points, divided by total number of days	Quantifies average movement rate
Mean cos angle	M	degrees	Calculated as the ratio of hypotenuse to adjacent side (both in km)	Detect directionality in movement path
mcparea (minimum convex polygon area)	M	km^2	Minimum convex hull fitted to all points; area calculated in degrees using Matlab's routine; degrees converted to distance using deg2km of square root	Traditional home range estimator; gives a simple measure of area used
nDBclusters nDBoutliers	M	N	Number of clusters and outliers as determined by DBSCAN algorithm (http://code.google.com/p/dmfa07/downloads/detail?name=DBSCAN.M) using at least 24 points per cluster (i.e. 3 days of data) and undetermined epsilon (neighbourhood distance).	Measures finer-scale cluster structure of data, particularly whether bird spends most of its time in one location or regularly undertakes wandering/exploratory movements
determinant	M	–	Take the determinant of the covariance matrix of the lat-long vectors	Measure of dispersion
Meanfndist Mean Nndist SDNNDist	M	km	Calculate distance from each point to its furthest neighbour and nearest neighbour; use the mean, of these values. We performed the same calculation for nearest-neighbour distances and included the standard deviation of the nearest-neighbour distance	Measure of dispersion
Pointdensity	M	Points/ mcparea	Divide total number of points by area of minimum convex polygon	Another way of measuring spatial dispersion

further each day and further during the course of the study than Egyptian geese in the other two sites, although at a considerably lower average speed than Egyptian geese from Barberspan. This difference can be partially attributed to the statistical influence of the fast, long-distance moult migration undertaken twice a year by most of the Barberspan birds. By contrast, red-billed teal appeared to move in similar ways across all three sites, although individuals tagged at Lake Manyame had slightly higher average movement rates and distances than did birds from the other two sites.

Although we did not know the sex of tagged birds at the time of PTT attachment, we later sexed most of the birds successfully using molecular methods. For the red-billed teal, 2 of the 10 birds analysed here were female, 1 was undetermined, and 7 were male. For the 16 Egyptian geese considered in the analysis, 7 were male, 1 undetermined, and 8 female.

For the movement variables, the first three principal components captured over 80% of the variance in the data (respectively, 49.7%, 22% and 12%). For the confounding data, two components were sufficient to explain over 90% of the variance (respectively, 66.15% and 24.9%). The first principal component of the movement data (movement PC1) was (only just) not significantly correlated with the first principal component of the confounding data (Spearman's $\rho = 0.39$, $P < 0.052$). When compared with the original predictor variables, movement PC1 appeared to capture elements of broad-scale home range size and use intensity, correlating significantly with maximum daily movement rate, minimum convex polygon (MCP) area, point density, determinant, mean furthest and nearest-neighbour distances, and the standard deviation of nearest-neighbour distance (Spearman's $\rho > 0.56$ in all cases, $P < 0.05$).

A boxplot of movement PC1 by site for each species (Fig. 3) supports our subjective impressions that: (1) Egyptian geese at Barberspan (latitude 26° S) are behaving in a different way from those at Strandfontein (latitude 34° S); (2) overall, red-billed teal show little difference in movement patterns between sites; and (3) Egyptian geese and red-billed teal are moving in similar ways to one another at both ends of our latitudinal transect but not at Barberspan. To test these conclusions more rigorously (i.e. with full correction for all potentially confounding factors) will require a larger sample size, but this analysis nonetheless provides some valuable insights.

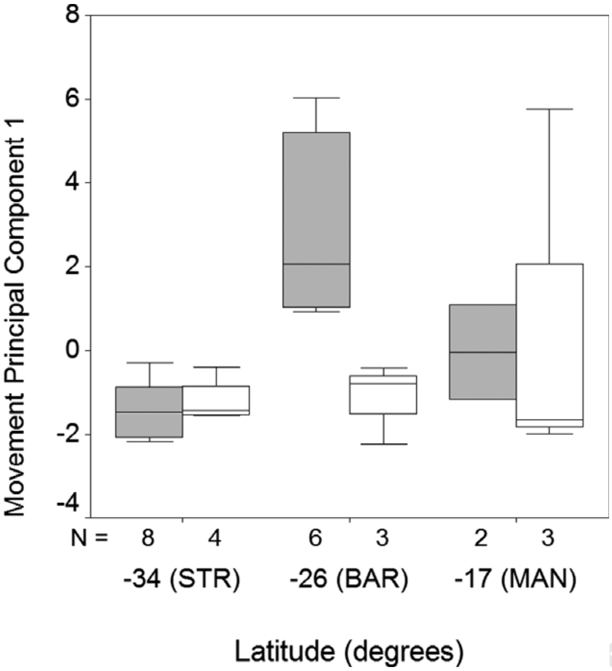

Figure 3 Boxplot of means and standard deviations by site and species (shaded grey for Egyptian geese, *Alopochen aegyptiaca*; white for red-billed teal, *Anas erythrorhyncha*) for the first principal component derived from the movement variables summarized in Table 2. Each pair of boxes is labelled by degrees of latitude and site. The smaller numbers just below the category (x) axis indicate sample sizes. Site abbreviations: BAR, Barberspan; MAN, Lake Manyame; STR, Strandfontein Wastewater Treatment Works.

DISCUSSION

Case study results

Several interesting and unexpected results emerged from this analysis. Red-billed teal appear to move with a relatively high degree of consistency at each of our three study latitudes, while Egyptian geese show latitude-dependent variation in their movements. However, much of the latitude-related variation for Egyptian geese appears to be driven by the long distances that Barberspan birds move to moulting sites and back to their 'home' areas, rather than directly by fluctuations in resource availability (especially given that Barberspan is surrounded by farms at which food for a grazing bird is plentiful during much of the year). None of our study years was a drought or flood year and, in addition, birds were moving into our most variable rainfall region, rather than out of it, in order to moult. Our results thus imply that, in normal years, range expansion may be limited by fidelity to moult sites (or at least to moulting areas). Internal drivers of movement, and particularly those relating to the life history requirements of breeding and moulting, appear to dominate over external drivers of movement for both species.

Knowledge from prior experience of the distribution of resources may influence waterfowl dispersal strategies and their movement responses to wetlands and resource distribution (Roshier *et al.*, 2001, 2008; Nathan *et al.*, 2008). The influence of experience, a question of navigation capacity, is commonly expressed by fidelity to breeding or moulting sites in long-distance migratory birds and to roosting sites at a finer scale (De La Cruz *et al.*, 2009; Guillemain *et al.*, 2010). The results from Egyptian geese must be interpreted within the

Table 3 Selected summary statistics by site for each bird species. Sample sizes are $n = 16$ for Egyptian geese (*Alopochen aegyptiaca*) and $n = 10$ for red-billed teal (*Anas erythrorhyncha*). A full set of summary data for each individual bird is presented in Appendix S1. Means and standard deviations were calculated across all birds of a given species at a given site; so, for example, estimating the mean of the maximum daily movement rate involves: (1) taking the highest movement rate during the study for each individual bird; and then (2) calculating a mean value for all birds from that site. Site abbreviations: BAR, Barberspan; MAN, Lake Manyame; STR, Strandfontein Wastewater Treatment Works. We have not provided standard deviations for MAN Egyptian geese because $n = 2$.

Species	Metric	BAR	MAN	STR
Egyptian goose	Number of tracked birds considered in this analysis	6	2	8
	Mean (SD) of distance moved during study (km)	7425 (2303)	9542	2664 (1737)
	Mean (SD) kilometres per day	9.65 (4.3)	12.05	4.5 (1.9)
	Mean (SD) [between individuals] of maximum daily movement rate	1537 (173)	885	505 (230)
Red-billed teal	Number of tracked birds considered in this analysis	3	3	4
	Mean distance moved during study (km)	2617 (1524)	1977 (605)	2024 (427)
	Mean kilometres per day	5.43 (0.4)	6.57 (2.3)	4.05 (1.8)
	Mean (between individuals) of maximum daily movement rate	383.24 (350)	632.0 (625)	492.49 (135)

context of an increasing and range-expanding population (Okes *et al.*, 2008). Our results from Barberspan suggest that, despite their relatively recent range expansion into the Eastern and Western Cape, Egyptian geese have retained a conservative moulting strategy.

Several other studies of wildfowl movement have also found a high degree of individual variability in movement patterns in response to similar environmental conditions, suggesting that complex behavioural strategies at the individual level interact with resource distributions to produce multiple movement patterns (Roshier *et al.*, 2008; Oppel *et al.*, 2009). Our comparison of movement patterns of two representative duck species along a latitudinal gradient thus suggests, somewhat surprisingly, that for Afrotropical ducks, many of the drivers of movement may relate more closely to 'internal' demands (such as prior experience and moult site requirements) than to the 'external' drivers of rainfall and food availability that are more commonly evoked in accounts of the ecology of waterbirds (e.g. Herrmann *et al.*, 2004; Chambers & Loyn, 2006). In other words, longer-term adaptation in the form of individually resilient patterns of responses to the variable, arid southern African environment appears to be the dominant variable shaping the biogeography of Afrotropical anatids. We anticipate that further data collection, coupled with specific analyses of our data in relation to environmental variation and life history traits (e.g. spatial variation in breeding periods), will allow us to test competing hypotheses further and to build on these foundations.

GENERAL DISCUSSION AND CONCLUSIONS

As our case study results demonstrate, the relevance of movement ecology for understanding biogeographical patterns goes well beyond the simple limitation of species range expansions by habitat availability or obvious barriers to movement. The hypothesis that our two study species are more likely to be movement-limited than environmentally or history-limited (at an ecological level of explanation), even though they are not dispersal-limited in a classical sense, provides a different perspective on attempts to understand their current distributions and to model their potential responses to environmental change. Although our eventual sample size was not sufficient for us to test some of the hypotheses that we had hoped to address, our analysis demonstrates the potentially high value of satellite telemetry data sets and other detailed analyses of movement (e.g. ringing recoveries and stable isotope analysis of feathers) for testing biogeographical hypotheses and developing ecologically sound species occurrence models.

The next challenges in our ongoing research on the biogeography of these species will be: (1) to link movement patterns to spatial variation in the timing of different life history stages (particularly moulting and nesting); and then (2) to combine species occurrence models that capture habitat suitability and more dynamic movement models to try to understand when, where, and why our study species are moving and how these movements relate to concerns over hunting regulations, conservation goals, and the risks of spread of infectious diseases of wild birds and poultry (e.g. avian influenza, Newcastle Disease; see discussion in Gaidet *et al.*, 2010).

More generally, we have argued that one of the key objectives for the further development of biogeography is to achieve a better integration of movement ecology with biogeographical analysis. As our case study demonstrates, the various elements of movement must be unpacked and considered individually before we can claim to have understood the biogeography of a mobile species. Ignoring the subtleties of movement and focusing purely on environmental drivers of species occurrences may lead to a wide range of false assumptions and inaccuracies in predictive models. Explorations of the ways in which movement varies across broad-scale gradients, and attempts to understand and interpret species range limits in the context of detailed telemetry data, offer a fertile area of enquiry for future research.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Appendix S1 Supplementary data on tracked birds and their movements.

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VETERINARY RESEARCH

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Linking avian communities and avian influenza ecology in southern Africa using epidemiological functional groups

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Abstract

The ecology of pathogens, and particularly their emergence in multi-host systems, is complex. New approaches are needed to reduce superficial complexities to a level that still allows scientists to analyse underlying and more fundamental processes. One promising approach for simplification is to use an epidemiological-function classification to describe ecological diversity in a way that relates directly to pathogen dynamics. In this article, we develop and apply the epidemiological functional group (EFG) concept to explore the relationships between wild bird communities and avian influenza virus (AIV) in three ecosystems in southern Africa. Using a two year dataset that combined bird counts and bimonthly sampling for AIV, we allocated each bird species to a set of EFGs that captured two overarching epidemiological functions: the capacity of species to maintain AIV in the system, and their potential to introduce the virus. Comparing AIV prevalence between EFGs suggested that the hypothesis that anseriforms (ducks) and charadriiforms (waders) drive AIV epidemiology cannot entirely explain the high prevalence observed in some EFGs. If anseriforms do play an important role in AIV dynamics in each of the three ecosystems, the role of other species in the local maintenance of AIV cannot be ruled out. The EFG concept thus helped us to identify gaps in knowledge and to highlight understudied bird groups that might play a role in AIV epidemiology. In general, the use of EFGs has potential for generating a range of valuable insights in epidemiology, just as functional group approaches have done in ecology.

Introduction

The ecology of pathogens and the emergence of diseases in multi-host systems are complex [1,2]. Understanding epidemiology often requires the incorporation of a wide variety of different kinds of evidence and disciplinary approaches [3]. Traditional surveillance and control approaches have often focused on humans, domestic animals, and known vectors. However, an increasing body of information indicates that effective disease surveillance and control may be heavily dependent on understanding the epidemiology of pathogens in relation to the ecology of their wild hosts e.g., [4-7].

Avian Influenza Viruses (AIVs) in wild birds have recently received increased attention due to the emergence of the Highly Pathogenic AIV H5N1 strain and its potential threat to human health [8]. Although numerous studies of low pathogenic AIV strains (LPAI) in waterfowl and wild birds have been published, encompassing tens of thousands of sampled wild birds, we still know relatively little about the susceptibility of individual bird species to AIV in relation to the global number of bird species [7]. The avian community in a single ecosystem can include hundreds of interacting species. In addition, the response of bird species to specific AIV subtypes (16 hemagglutinins and 9 neuramidases known) is variable and prevalence patterns of specific subtypes will be determined by the bird cenosis. So far, most studies of AIV have concentrated on anseriforms and to a lesser extent on charadriiforms, which are known to be reservoirs for LPAI [7,9]. In their synthesis of wild bird low pathogenic avian influenza surveillance worldwide, Olsen et al. [7] found that out of more than 90 000 birds sampled, 54% were anseriforms and 25% charadriiforms. As a consequence, little information on AIV prevalence in the rest of the avian community has been published,

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and much of what has been published has been obtained as “by-catch” from capture protocols that have been focused on ducks. The minimum sample sizes that would be necessary to confidently estimate prevalence for most non-target bird species are often not reached, with the risk that the common practice of focusing mainly on anseriforms may be overlooking the role of other bird groups in the epidemiology of AIV in waterfowl communities.

Biases in the selection of species to sample are not the only problem in available data sets for AIV. The comparison between sample and community composition is a fundamental parameter in epidemiological studies [10]. In many cases, a lack of information regarding the composition of the wild bird community from which the sample is taken makes conclusions from AIV studies even harder to interpret. A total of 100 positive samples from mallard ducks (*Anas platyrhynchos*), for example, carries quite different epidemiological implications if mallards represent 0.1% versus 90% of the number of wild birds present in the ecosystem; and similarly, the relevance of 100 positive samples from mallards differs if the system contains 10 or 100 other species. Interpretation of the role of a species in pathogen maintenance cannot be done rigorously without considering the potential role of the rest of the community. The sampling bias that is attendant on any field captures of wild birds should therefore be a crucial parameter in wildlife epidemiological studies.

As more host species are considered in a host-pathogen system, the number of potential interactions (and hence, the complexity of potential pathogen transmission pathways) increases exponentially in relation to the number of species in the analysis. The problem is further complicated, in the case of AIV, by the existence of a diversity of viral subtypes and substantive variations in pathogen-host interactions (e.g. susceptibility and pathogenicity). It rapidly becomes both empirically and computationally unfeasible to analyse the specific relationships between each host species and each pathogen subtype; and assigning each host species to a specific role in the epidemiological cycle (e.g. reservoir, dead end-host, spreader) can be extremely difficult even when large, detailed data sets are available. Often, in such contexts, management and policy responses must nonetheless occur. The control of emerging pathogens, for instance, typically requires rapid responses that are based on partial and imperfect information. There is therefore a clear need for techniques that can be used to summarize epidemiological complexity without oversimplifying it, even if resulting conclusions are later modified by the findings of more intensive studies.

This kind of problem, in which the number of interacting elements and interactions rapidly exceeds what can reasonably be measured in a typical scientific study, is common in research on complex systems. For example, overwhelming complexity generated by multiple interacting influences is a unifying problem in research on systems as diverse as gene expression, stock markets, and ecosystems. Epidemiologists have generally responded to interaction complexity by simplifying the description of what constitutes the system, deliberately excluding potentially interacting members of the epidemiological network. While this approach has produced some successes, particularly in understanding specialist pathogens with simple transmission cycles and limited numbers of interacting hosts and vectors, it also carries some potentially serious weaknesses in both theoretical and applied realms (as highlighted by analyses of the boundary specification problem in network analysis; e.g. [11]).

An alternative approach to system simplification (i.e. rather than selectively picking out a small subset of interacting species to consider as “the system”) comes from the field of community ecology, in which researchers have attempted for decades to deconstruct the complexity of food webs [12]. Concepts such as trophic levels and foraging guilds have played an important role in the development of ecological theory; and Elton’s trophic pyramid, in which differences in the biomass of different trophic levels are explained by the second law of thermodynamics, is one of community ecology’s most fundamental generalisations. Many of the approaches that have been developed for food web analysis in ecology are readily applicable to the analysis of the ecology of pathogen transmission in multi-host systems [1,2,13,14].

The idea underlying functional group analysis is that broad, community-level trends in processes of interest can be detected by replacing a taxonomic classification of hosts with a classification that groups hosts according to their functional role in the epidemiology of a pathogen or a group of pathogens [15]. Although “AIV” describes a group of pathogens, we treat it as a single pathogen, ignoring AIV subtype variability, because the sparse information available does not suggest that AIV modes of transmission significantly differ among subtypes (see [16,17] for details). Hosts in an epidemiological functional group (EFG) share a common function in the epidemiology of the pathogen(s) of interest. We used the concept of EFGs to (1) investigate the ecology of AIVs in three different wild bird communities in southern Africa; and (2) critique the current scientific paradigm for field investigations of AIV in wild birds.

We used a wild bird census dataset to first allocate species to EFGs according to two epidemiological functions (reservoir vs. non-reservoir species, and the potential to introduce AIV strains through migratory behaviour; note that the functional groups, as described later, differ from the functions themselves) according to current AIV epidemiology dogma. We ranked each group in relation to their expected contribution to pathogen prevalence and then used our rankings to calculate relative *a priori* risk for each group. We then compared these *a priori* relative risks (which are effectively predictions, generated by accepted knowledge) to our empirical data on observed prevalence per group. In other words, we used EFGs as a way of exploring the degree to which empirical data match commonly held assumptions, rather than adopting the commoner approach of attempting to classify species into EFGs based on our own data. Our results suggest that commonly held assumptions may require some re-thinking.

Materials and methods

Study sites

We undertook research on bird communities at three geographically distinct sites in southern Africa. (1) Barberspan Nature Reserve (BAR) in the North West Province of South Africa, is a RAMSAR wetland of total area varying between 1000 and 1700 ha (GPS coordinates 26° 35’00”S, 25°35’30”E); (2) Strandfontein wastewater treatment works (STR) in the Western Cape Province, South Africa, is a 319 ha water body located near Muizenberg on the immediate periphery of the city of Cape Town (GPS coordinates 35°05’00”S, 18°30’45”E); and (3) the Manyame-Chivero dams (MAN) in Zimbabwe, which are man-made impoundments that are linked by the Manyame river and were built in the 1950s to supply the city of Harare with water (GPS coordinates 30°30’30”, 17°45’00”). They cover areas of 6500 and 18 500 ha respectively. More information on the sites is available as supplementary material in Cumming et al. [17]. We selected our study sites based on three main criteria: (1) their designations as Important Bird Areas (as recognised by Wetlands International) with a high ornithological diversity; (2) the location of sites along a latitudinal gradient; and (3) their feasibility as study sites, which in this case meant finding a good compromise between the first two criteria, the need to sample each site at a high frequency, and the constraints imposed by available financial and human resources.

Baseline data

All necessary permits to undertake this study were obtained from the relevant authorities; at STR, from the Cape Nature and Cape Town City Council; at BAR, from the North Wets Parks and Tourist Board; at MAN from the Governmental Veterinary Services and the Park and Wildlife Management Authority from Zimbabwe.

Bird census data were collected, using standardised point counts, from February 2007 to May 2009. Each point count consisted of a 10-min habituation period followed by a 30 min focal count of all birds in a semicircle of 150m radius, facing the waterbody. Point counts were undertaken at 12 to 15 points at each of our three sites (BAR, STR and MAN) and were repeated four times at each location over five days during each counting and sampling session. Sessions were repeated every two months [17].

The prevalence of AIV (“estimated prevalence”) was estimated by capturing and sampling birds at each site every two months, over the two-year period from February 2007 to March 2009. Capture sessions were undertaken during a week-long intensive sampling period immediately after each 5-day counting session. Wild birds were caught using walk-in traps, mist nets and with occasional use of spring- or cannon-nets placed near the water’s edge. Diagnostics were performed using a real-time reverse transcription PCR technique on cloacal and tracheal swabs stored in a viral transport medium (Hank’s salt solution with antibiotics and fungicides) and kept in liquid nitrogen containers before delivery at the laboratory. Additional details on the protocols have been published in Cumming et al. [17].

Initially, the sampling protocol implemented in the three study sites was designed according to current knowledge about AIV epidemiology in wild birds. As almost no information was available from southern African ecosystems on AIV, it was decided to test the assumption that anseriforms and to a lesser extent charadriiforms were the main reservoirs of AIV in southern Africa. Capture methods were therefore chosen to maximize duck captures. All “by-catch” species were also sampled for AIV.

Data analysis

Our analysis followed four main steps: (1) allocating bird species to EFGs, based on known characteristics of AIV ecology in wild birds, and assessing semi-quantitatively the risk associated with each EFG; (2) comparison of the waterfowl communities’ characteristics across the 3 sites by using biodiversity indices; (3) comparison of how representative the bird sampling was of the observed avifauna at each site; and (4), integrating the information gathered through the comparison of waterfowl communities, comparison between wild bird communities and composition of the bird captured sample and the estimated prevalence for each EFG in each study site to explore the relevance of the current AIV in wild bird dogma in these three ecosystems. Although we did obtain time series of prevalence, the temporal data exhibit high levels of variation and we have not included them in this manuscript for the sake of clarity (see [16,17] for detailed analyses).

Allocating bird species to EFGs

In order to reduce the complexity of the multi-host systems studied (several hundred bird species in each ecosystem), we defined two epidemiological functions (EF), with relevance to AIV epidemiology, on the basis of which we could allocate host species to EFGs. Hosts can play a limited number of roles (e.g., reservoir, dead-end host) in the epidemiology of a pathogen. Epidemiological functions related to each role can thus be used to regroup hosts into EFGs. Our initial assumption was that host species belonging to the same EFG would share more AIV epidemiology-related traits than host species in other EFGs. The two epidemiological functions (EFs) that we considered were (1) the maintenance and (2) the introduction of AIV strains. Note that for each epidemiological function, several different epidemiological functional groups exist. Both EFs reflect current and mainstream understanding of AIV ecology in wild birds.

The first EF relates to the AIV maintenance capacity of different bird species. The target population (according to the definition of [2]) is at risk of AIV transmission from the maintenance population either directly or indirectly through the non-maintenance population. The anseriforms and charadriiforms are bird orders that are considered globally to be reservoirs for AIV, and many studies consider only these two orders for epidemiological investigations (e.g. [18,19]). If there is an endemic AIV cycle in southern Africa, we hypothesized that anseriforms and potentially charadriiforms would constitute the maintenance community. We allocated anseriforms and charadriiforms into two different EFGs because they usually do not share either the same AIV prevalence or the same subtype pool, and do not always share transmission pathways [7]. In Africa, a role as a reservoir for both groups has been suggested by recent studies [16,17,20,21]. The other bird orders have not been sufficiently investigated to assign them different roles in viral maintenance. According to currently accepted assumptions about AIV ecology in wild birds, non-anseriform and non-charadriiform species are not associated with particular roles in AIV epidemiology. We thus grouped all of these species into the same EFG. This resulted in three epidemiological functional groups for AIV maintenance: *Ans* (anseriforms), *Cha* (charadriiforms) and *RoC* group (Rest of Community), with the latter category containing all non-anseriform and non-charadriiform bird species. If anseriforms and charadriiforms represent the main reservoir of AIV in southern Africa, the *RoC* group should play a minor role in the ecology of AIV, with occasional spill-over of AIV strains triggering infections, and the estimated prevalence in this group should thus be lower than in the two other groups across the study. Anseriforms usually present a higher AIV prevalence than charadriiforms [7]. Based on this information, relative risks of 3, 2 and 1 were allocated to *Ans*, *Char* and *RoC* respectively. Values allocated here are semi-quantitative and should be considered simply as ranking the expected prevalence for each group, rather than as describing its relative magnitude.

The second EF concerns the potential of bird species to introduce AIV strains into the ecosystem from different ecosystems across regions or continents. As birds move or migrate away from a given ecosystem, they will be exposed to a greater variety of AIV strains and could introduce those strains into the ecosystem on their return. The role of migrating wildlife in the spread of diseases has been recently reviewed [22]. Although long-distance migration is not systematically correlated with pathogen dispersal, the role of wild birds in spreading LPAI has been documented [23,24]. Depending on the circulation of AIV in the ecosystem under study, the introduction of exogenous strains could trigger epizootics if no cross-immunity against these strains exists. Such introductions could also play a role in reassortment processes and the emergence of new strains [25,26]. We thus allocated birds in our study communities to the following epidemiological functional groups relating to pathogen introduction: (a) Long range spreader or Palaearctic (*Pal*) migrant, migrating from Eurasia where a higher prevalence of AIV can occur at some times of year [27]; (b) Middle range spreader or Afrotropical (*Afr*) migrant, migrating North of the equator in Africa; (c) Local spreader or nomad (*Afr*, see discussion below), moving regionally to follow resources and/or undertake moult or breeding-related local migrations; and (d) Non spreader or Resident (*Res*) bird with limited local movements.

Despite the availability of detailed information about wild bird ecology in southern Africa [28], the movement ecology of many species remains unclear, particularly when geographically distinct populations of the same species behave differently (e.g., [29]). We therefore decided to group medium and local-scale spreader species into a single *Afr* (mobile Afro-tropical) group. A role for Palaearctic birds in the introduction of Eurasian AIV strains in Africa has been suggested [30,31]. If there is no endemicity of AIV in southern Africa, we hypothesized that Palaearctic migrants should introduce AIV regularly in these ecosystems. By contrast, a community dominated by the “Resident” EFGs should experience little AIV circulation. Based on these assumptions, relative risks of 3, 2 and 1 were allocated to *Pal*, *Afr* and *Res* respectively.

We then combined both EFs by creating a matrix of 3×3 EFGs as used for the analyses. The relative risk at the EFG level was calculated by multiplying the relative risks of each EF (Table 1), providing a ranking of AIV

Table 1 Epidemiological functional groups and relative risk

EF2 EF1	Anseriforms *Ans = 3*	Charadriiforms *Cha = 2*	Rest of Community *RoC = 1*
Resident *Res = 1*	*Ans-Res = 3*	*Cha-Res = 2*	*RoC-Res = 1*
Afro-tropical migrant *Afr = 2*	*Ans-Afr = 6*	*Cha-Afr = 4*	*RoC-Afr = 2*
Palaearctic migrant *Pal = 3*	*Ans-Pal = 9*	*Cha-Pal = 6*	*RoC-Pal = 3*

Epidemiological functional groups used in this study, based on the two epidemiological functions related to the maintenance and introduction potential respectively of AIV in Southern African ecosystems. Numbers represent qualitative estimations of the AIV relative risk for each epidemiological function and for each EFG. For each cell, the qualitative estimation of the relative risk is calculated by multiplying values of the relative risk of EFGs from EF1 and EF2.

prevalence between groups to be challenged by field data. Risk values were multiplied, as is the norm for probabilistic estimates of risk, because group scores captured the non-linear interactions between EF1 and EF2 risks.

Chi-square tests were used to compare prevalence between sites and EFGs. Spearman Rank Correlation tests were performed to compare proportion of observed vs. captured EFGs and semi-quantitative variables of risk estimation.

Comparison of bird communities between sites and bird sampled vs. counted

Two indices were calculated to describe the waterfowl community in the three sites: species richness (total number of species) and Shannon's diversity index [32], which combines species richness and abundance. The influences of both host richness and abundance have been shown to be important for epidemiological dynamics [33]. Both indices were calculated across the two years of count data. The bird species of the 3 sites were allocated to the 9 EFGs using available regional knowledge [28] and the composition of these groups was compared across ecosystems (see below). The relative bird density was represented by the total number of birds observed divided by the total number of counts for that site; no additional correction for area was necessary because all counts were undertaken within a semicircle of 150m radius.

We estimated how well our sampling represented the observed bird community and the bias induced by the bird capture techniques and the "catchability" of waterfowl species by comparing the proportions of each bird group captured and observed across the two years of capture. A Spearman rank correlation test was performed between observed and captured birds in each of the three sites in order to assess how representative the sample composition was *vis a vis* the observed bird community.

Prevalence and AIV risk estimation for EFGs

For each EFG and for each site, we calculated the estimated prevalence by dividing the number of positive birds by the number of birds sampled. We also estimated the "*a priori* risk" of AIV by multiplying the relative risk presented in Table 1 by the EFG proportions observed (from the total counts) in each different ecosystem. We assumed a similar weight for both maintenance and introduction functions. These "*a priori* risk" values provide semi-quantitative predictions about AIV circulation in each of the EFGs according to current knowledge of AIV in wild birds.

We then calculated an "estimated risk" by multiplying the AIV prevalence calculated at the EFG and study site level by the EFG proportions observed (again from the total counts) in each of the ecosystems. The "*a priori* risk", which captures current knowledge about AIV epidemiology, was then compared to the "estimated risk", which captures observed prevalence and community composition in our three sites in southern Africa. For both risks, we summed the risk value across EFGs to calculate a "Site community" value, which is an estimation of both risks at the study site level. We compared the "*a priori* risk" with the "estimated risk", excluding the Resident and Palaearctic anseriforms that were absent from all three ecosystems.

Results

Comparison of waterfowl communities observed and captured between sites

The bird community at MAN had higher species richness, a higher Shannon index and a lower relative bird density than STR and BAR (Table 2). STR was less diverse (139 against 199 species recorded) and the values of the Shannon index were smaller than in BAR. The avian community composition relative to EFGs across the three sites differed (Table 2). BAR and STR were dominated by *Roc-Afr*, MAN by *Ans-Afr.* In all 3 ecosystems, *Ans-Afr* and *Roc-Afr* represented more than 17% of the birds observed and there were no *Ans-Res* and *Ans-Pal* and only a few *Roc-Pal*. Few *Cha-Pal* (between 2.6 and 5.1% of all counted birds) were present in these ecosystems. MAN, STR and BAR did not differ in anseriforms (mainly Afro-tropical migrants) density, but did differ in the relative abundance of anseriforms in the total community (Table 2, Figure 1).

In all three sites, the *Ans-Afr* EFG was over-represented in the sampled birds (Figure 1), reflecting the objectives of the sampling protocol. In addition, some bird families that were abundant in the counts were poorly represented or absent from the captured birds: the *RoC-Afr* group for all three sites and *Char-Afr* in STR. No *Cha-Pal* were captured at STR. Only in BAR, the captured and observed EFGs were correlated (STR: Spearman's r = 0.37, p = 0.497), BAR: Spearman's r = 0.92, p < 0.010, MAN Spearman's r = 0.83, p = 0.058).

Table 2 Indicators of waterfowl community diversity

		BAR	STR	MAN
	Bird Obs/Count	246 ± 537	234 ± 216	144 ± 171
	Species richness	198	138	249
	Shannon's index	2.72	2.95	3.54
EFG	***Ans-Res***	0.0%	0.0%	0.0%
	Ans-Afr	**17.0%**	**19.4%**	**34.0%**
	Ans-Pal	0.0%	0.0%	0.0%
	Cha-Res	0.2%	8.2%	0.1%
	Cha-Afr	6.3%	**30.0%**	**17.3%**
	Cha-Pal	4.3%	2.6%	5.1%
	RoC-Res	6.8%	4.5%	**14.4%**
	RoC-Afr	**65.1%**	**34.6%**	**27.1%**
	RoC-Pal	0.3%	0.7%	2.0%
	Total	100.0%	100.0%	100.0%

"Birds Obs/Count": average number of birds observed per count and standard error displayed; "Species richness": number of species observed across the two years; "Shannon's index" diversity index. Proportions of each combined epidemiological functional group (EFG) are displayed in each ecosystem (Ans = anseriforms, Cha = charadriiforms, RoC = Rest of Community, Res = resident, Afr = afro-tropical, Pal = palaearctic). In bold, dominant EFGs for each ecosystem.

AIV prevalence and risk comparison for EFGs

The anseriforms afro-tropical group represented the only anseriforms present in all three sites and their AIV prevalence was 1.1, 1.2, and 5.0% respectively for BAR, STR & MAN (95% Confidence Intervals being [0.7:1.9], [0.4:1.7], [4:5.9] respectively) differing significantly between MAN and both BAR and STR (both chi-square tests being highly significant, p < 0.001). *Cha-Res*, *Cha-Afr* and *Cha-Pal* had zero prevalence at both BAR and STR, albeit with small sample sizes (maximum possible prevalence 98.0%, 2.8%, 8.2% for BAR and 9.0%, 7.8% and NA for STR respectively for the 3 EFGs at 95% Confidence Interval). At MAN, *Char-Afr* had a relatively high AIV prevalence (as for *Char-Pal*) but with a large confidence interval (Figure 2). The *RoC-Afr* group had

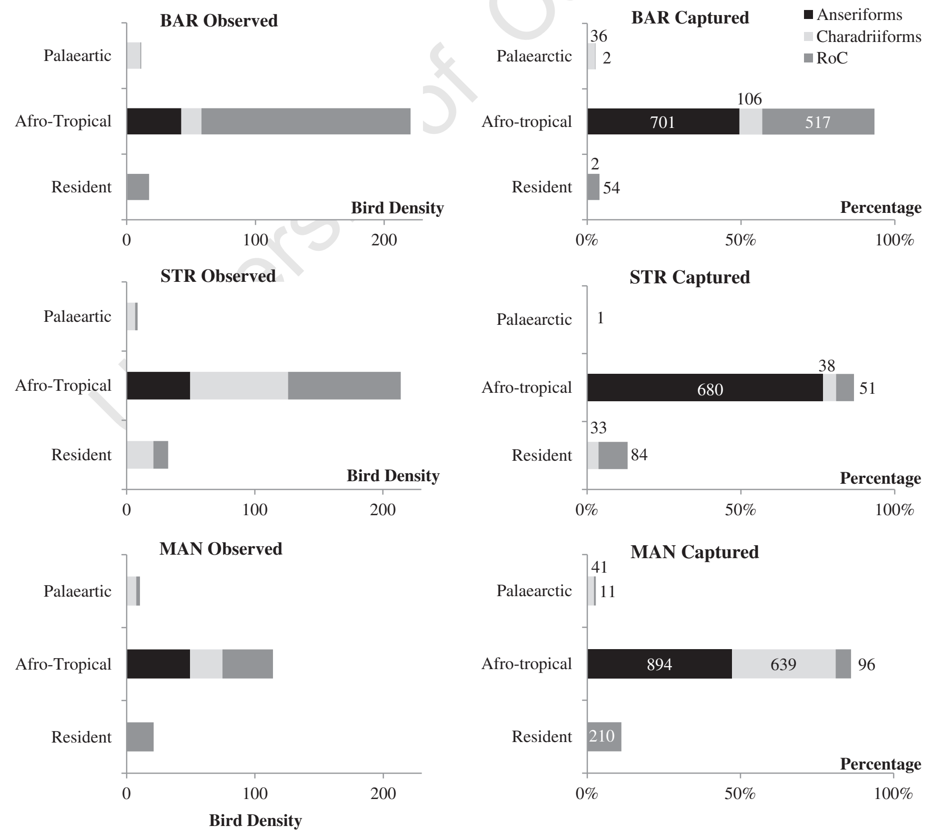

Figure 1 Community observed and captured in the three sites. Community observed (left) and captured (right) in the three sites according to EF 1 & 2 groups. Bird density ("Observed") is calculated by the number of birds observed divided by the number of counts (counts implemented in a given area). Bird abundance ("Captured") is the pecentage of birds captured (numbers indicate the number of birds captured per EFG). Dark grey = anseriforms, Medium grey = RoC and Light Grey = charadriiforms.

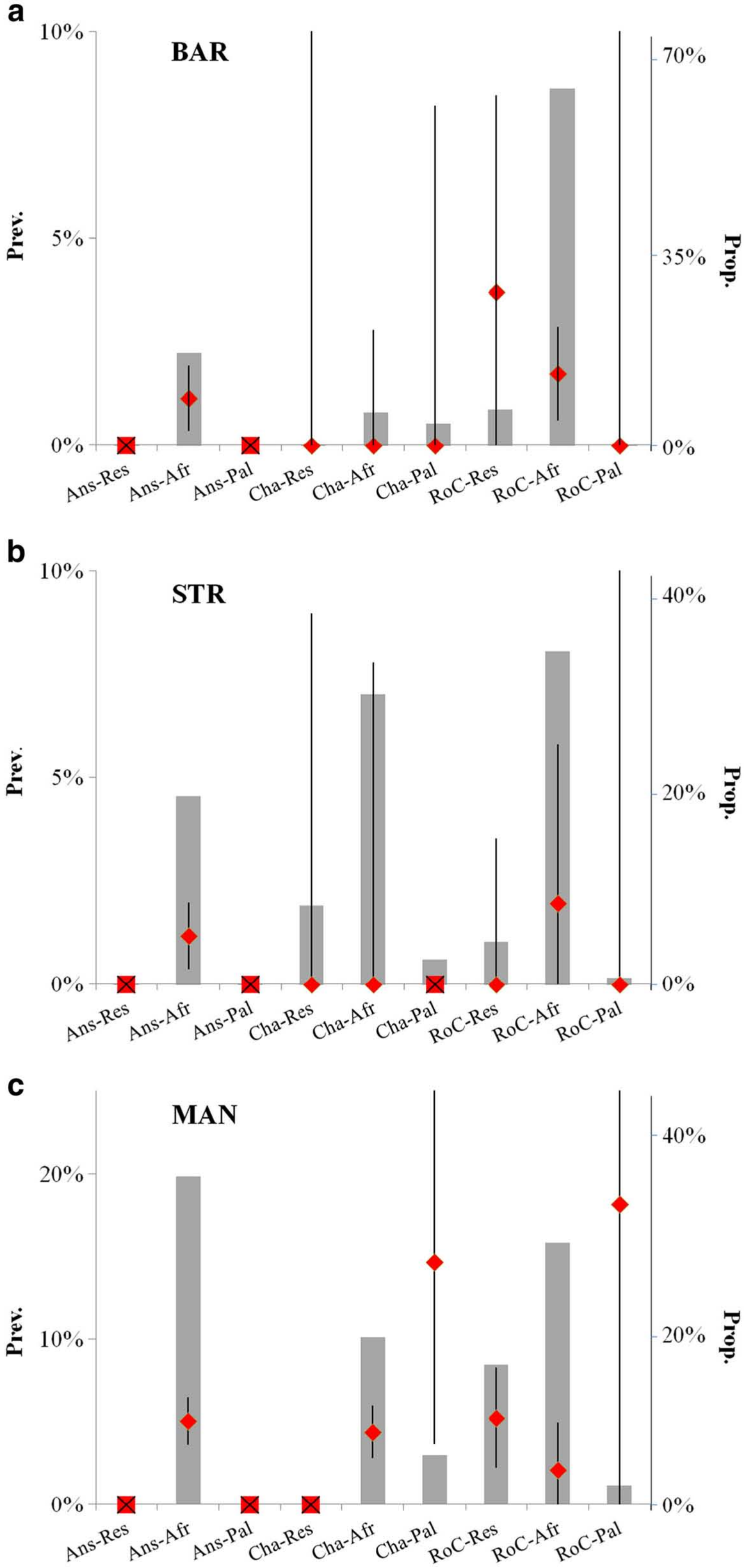

Figure 2 AIV Prevalence for each EFG in relation to bird community composition in the three sites. For each site (BAR, STR, MAN): **a**) diamonds represent AIV prevalence with 95% confidence interval (left axis) for each combination between EF1 & EF2 (Ans = anseriforms, Cha = charadriiforms, RoC = Rest of Community, Res = resident, Afr = afro-tropical, Pal = Palaearctic migrant); **b**) grey bars represent proportion of each bird group in the bird community observed (or counted) during the 2 years of the project (right axis).

detectable prevalence in the three sites, *RoC-Res* for BAR and MAN and *Roc-Pal* had a high prevalence in MAN but with a small sample size. Any bird groups representing more than 15% of the community had detectable prevalence of AIV in all three sites with the exception of *Cha-Afr* in STR (but with only 38 individuals sampled).

All groups present in MAN had a detectable mean prevalence. The MAN prevalence for *RoC-Res* and *RoC-Afr* were significantly higher than BAR *RoC-Afr* (chi-square test, $p < 0.01$ and $p < 0.05$ respectively) and higher but not significantly different from BAR *RoC-Res* and STR *RoC-Afr* (also the sample size of this group was small). The prevalence of the *RoC-Afr* and *Cha-Afr* groups in MAN were not significantly different from the *Ans-Afr* group. In BAR and STR, AIV prevalence in well-sampled groups appeared similar.

According to "*a priori* risk" estimation based on current AIV in wild bird knowledge and the bird community composition, we expected AIV site prevalence to be higher in MAN, followed by STR and lastly BAR ("Site community" row in Table 3). The "estimated risk" using field prevalence and bird community composition also predicted a difference between the sites, with MAN having the higher risk estimation, followed by BAR and STR. This site level approach can be compared with an alternative hypothesis, based on current understanding of AIV epidemiology in wild birds. Without taking into account the bird community composition, we would expect a similar AIV prevalence in the three ecosystems because the density of anseriforms was similar in the three ecosystems (Figure 1, *Ans-Afr* = 42.5, 49.5 and 49.5 birds per counts/unit area for BAR, STR and MAN respectively). None of "*a priori* risk" and "estimated risk" were correlated (Table 3, for BAR, STR and MAN respectively, Spearman's r = 0.388, p = 0.396; Spearman's r = 0.449, p = 0.302; Spearman's r = 0.609, p = 0.167) indicating that *a priori* assumptions about the epidemiological role of the host community did not fit our field data.

Discussion

Analysing this epidemiological dataset in accordance with the current dogma of AIV in wild birds, anseriforms prevalence appears to drive prevalence at the community level in each ecosystem (Table 3) [16,17]. By including bird community data and the composition of the captured sample, and taking into account the EFG approach, we obtain a different perspective: (1) different bird communities predict different AIV risks ("*a priori* risk") using the EFG approach in the three ecosystems, a result validated by the "estimated risk" using field AIV prevalence; (2) sampling bias can explain discrepancies between the "*a priori* risk" and "estimated risk" for AIV prevalence; and (3) anseriforms play an important role in AIV epidemiology in waterfowl in the three ecosystems, as assumed by the current understanding of AIV ecology in wild birds, but other bird groups identified at the EFG level show unexpectedly high prevalence, and could play a role in the local epidemiology of AIV.

The EFG approach thus appears to be successful in improving our understanding of the role of wild birds in the epidemiology of AIV by highlighting potential epidemiological functions for unconsidered bird groups, identifying gaps in knowledge or sampling (see in Table 3, EFGs highlighted in italic) and suggesting new

Table 3 Sample size, estimated prevalence and relative risk for each epidemiological functional group

	BAR			STR			MAN		
	n	**Estimated Risk**	***A priori* Risk**	***n***	**Estimated Risk**	***A priori* Risk**	***n***	**Estimated Risk**	***A priori* Risk**
Site community	**1418**	**1.58**	**2.85**	***887***	**0.91**	**3.40**	***1891***	**4.9**	**3.64**
Ans-Res	0	na	0.00	*0*	na	0.00	*0*	na	0,00
Ans-Afr	701	0.19	**1.02**	*680*	0.23	**1.16**	*894*	**1.71**	**2.04**
Ans-Pal	0	na	0.00	*0*	na	0.00	*0*	na	0.00
Cha-Res	2	0.00	00.00	*33*	0.00	0.16	*0*	na	0.00
Cha-Afr	106	0.00	0.25	*38*	*0.00*	**1.20**	*639*	0.76	0.69
Cha-Pal	36	0.00	0.26	*0*	na	0.16	*41*	*0.75*	0.31
RoC-Res	54	*0.25*	0.00	*84*	0.00	0.00	*210*	0.76	0.00
RoC-Afr	517	**1.13**	**1.30**	*51*	**0.68**	0.69	*96*	0.56	0.54
RoC-Pal	2	0.00	0.01	*1*	0.00	0.02	*11*	*0.36*	0.06

Sample size, estimated risk and *a priori* risk for each epidemiological functional group in each of the three study sites. "Estimated risk" is the product of AIV prevalence calculated for each site across the 12 sampling sessions at the community level (row "Site community") and for each EFG and of the proportion of the EFG in the bird community (Table 2); n = number of birds sampled; based on results presented in Cumming et al. [17]; "na" indicates that no birds of this group were sampled. "A priori risk" for each EFG was globally calculated by multiplying the relative risk for each functional groups (Table 1) and the proportion of each group in the bird community (Table 2). Values in italic indicate groups that would require more sampling because of relatively high "a priori risk" or high "estimated risk" combined with small sample size. Values in bold for EFG indicate the highest respective values when sample size is adequate for both "estimated risk" and "a priori risk".

hypotheses. The EFG approach also carries the benefit of making better use of "by-catch" samples, which are often portrayed in AIV studies as secondary-level data [19].

We expected that different host community compositions in different ecosystems would lead to different epidemiological patterns. Our data show that bird communities differed substantially between sites and, as a consequence, the site "*a priori* risk" related to the two epidemiological functions varied (Tables 2 and 3). The larger size of the MAN ecosystem compared to the two other wetlands, and its more tropical location, may explain many of the observed differences in density and species richness. Some similarities in the bird communities were nonetheless observed across the sites. For instance, most anseriforms in southern Africa are afro-tropical species, as few Palaearctic anseriforms reach southern Africa. Other Palaearctic migrants (e.g. ruffs *Philomachus pugnax* and common sandpipers *Actitis hypoleucos*) arrive in the region from Eurasia in late September and early October, but most are present in relatively low numbers (Figure 1). The "*a priori* risk" of AIV introduction through intercontinental migration is therefore low compared to the same risk through intra-continental movements as most of the birds at our study sites remain within the afro-tropical region (88.4, 84.0 and 78.4% for respectively BAR, STR and MAN). The avian community composition at MAN appeared to be more favourable to AIV maintenance because it is dominated by afro-tropical anseriforms. At STR, the large presence of afro-tropical charadriiforms also suggested the possibility of AIV maintenance. At BAR, the *RoC* group dominated and we expected little AIV circulation (Table 3).

Our field data for AIV prevalence partially supported the "*a priori* risk" estimation based on current AIV ecology dogma. However, they did not support the hypothesis of equivalent AIV prevalence in the three ecosystems, despite similar densities of anseriforms. This suggests that sampling anseriforms without taking into account the rest of the bird community can lead to false conclusions. MAN has a higher AIV prevalence at the community level compared to BAR and STR, as indicated in Figure 2. However, the prediction that STR would have a higher "*a priori* risk" than BAR (Table 3) was not corroborated by observed "estimated risk". Our principal explanation for this discrepancy is the bias in the captured sample compared to the bird community composition at STR (Spearman rank correlation test). STR is the only site with a non-significant correlation coefficient between observed and captured bird community composition. We would expect that increased sampling in abundant EFGs such as afro-tropical charadriiforms and *RoC* groups at STR would increase our estimate of its site AIV prevalence and its "estimated risk". At BAR and MAN, the contribution of each EFG to the AIV prevalence is in agreement with field predictions in EFGs with a large sample size (i.e. more than 100 birds sampled).

Current knowledge about global AIV epidemiology applies to some extent to AIV epidemiology in these three southern African ecosystems. Notably, the important role of afro-tropical anseriforms in the epidemiology of AIV is confirmed in southern Africa [16,17]. However, higher than predicted AIV prevalence in other bird groups challenges the hegemony of anseriforms as the primary actor in the maintenance of AIV in these ecosystems. Firstly, the AIV prevalence estimated in the resident *RoC* group in BAR and MAN is not significantly different from the prevalence in the afro-tropical anseriforms group across the two years of the study. The same observation can be made for afro-tropical charadriiforms and to a lesser extent for the Palaearctic *RoC* group (with a high prevalence but a small sample size leading to a high maximum undetectable prevalence) at MAN. For all three ecosystems, the majority of AIV infected birds (estimated by multiplying the prevalence by the proportion of the group in the community in Figure 2) would not belong to the afro-tropical anseriforms group, contrary to what might be expected for the reservoir of the disease. In BAR and STR, for example, the majority of the infected birds would belong to the afro-tropical *RoC* group. Finally, all EFGs but one that represent more than 15% of the community have a detectable prevalence, suggesting a frequency-dependent role of EFGs in relation to AIV prevalence. A role for these EFGs in the persistence or maintenance of AIV in our study ecosystems cannot be ruled out, even though this conclusion would go against current dogma surrounding AIV epidemiology in wild birds [34]. Most studies of AIV ecology in wild birds have been implemented in temperate climates [7]. The current dogma in this field therefore comes from studies implemented in specific biophysical conditions (e.g. climatic condition, ornithological diversity) that have been shown to influence the epidemiology of AIV [35]. Often, this dogma is taken for granted when studies are implemented in other regions with different biophysical conditions. Our results indicate that more attention should be given to designing local hypotheses in reference to global assumptions: some space should be left for alternative hypotheses and the inclusion of count data and the sampling of other bird species than anseriforms and charadriiforms will serve this purpose.

The resident *RoC* groups represent more than 100 species in each ecosystem. Most of the species in these groups had no positive sample and a small sample size. A few species drive prevalence estimates at the group level but lack an adequate sample size. Cumming et al.

[17] identified some of the families or species that are high priorities for further sampling: for example, Hirundidae (1 positive out of 8, 7.7%), Alaudidae (3 out 24, 12.5%), and Motacillidae (2 out of 43, 4.7%). For some terrestrial passerine species, experimental data suggest a potential role in virus shedding (e.g. [36-39]). Too few samples from Palaearctic species were obtained through this study to provide a clear picture of their role (n = 2, 1, and 44 respectively for BAR, STR and MAN with only 8 positives in MAN). However, the 17% prevalence estimated for Palaearctic charadriiforms in MAN (n = 35) indicates the need for more information about this group.

This study was implemented to provide the first longitudinal AIV information for these ecosystems. Its initial design was similar to most wild bird AIV surveys, focusing primarily on anseriforms and charadriiforms that were assumed to play an important role in AIV epidemiology in all ecosystems. The sampling of all birds captured during the protocols allowed us to investigate additional hypotheses about AIV ecology in wild birds. What is more, by adding bird count data, we were able to combine our sampling and prevalence data with available ornithological knowledge to allocate bird species into EFGs and thus to simplify the multi-host complexity of the study system. Our data support the idea that some EFGs play an important role in the persistence and/or maintenance of AIV in southern African ecosystem. They also imply that comparisons of prevalence data from multiple sites (even if the sampling was done at similar time) are compromised if environmental and ecological variability is not accounted for. To understand key issues such as HPAI strain emergence and local maintenance of AIV, the role of the avian community as a whole must be considered; selective sampling of a largely unjustified subset of species from within an extensive interacting host community can no longer be justified. In general, our analysis provides strong support for the argument that functional approaches to complex host-pathogen systems can offer valuable ways of reducing the complexity of interactions to a more manageable level.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

AC contributed to conception and design of the study, data collection in Zimbabwe and provided the first and subsequent drafts of the manuscript. MdGW contributed to conception and design of the study and commented substantially on several drafts of the manuscript. MN contributed to conception and design of the study, data collection in South Africa and commented substantially on several drafts of the manuscript. GSC contributed to conception and design of the study, data collection in South Africa and commented substantially on several drafts of the manuscript. All authors read and approved the final manuscript.

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Host associations, biogeography, and phylogenetics of avian malaria in southern African waterfowl

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SUMMARY

The relevance of spatial variation in the environment and host communities for parasite community composition is poorly documented, creating a need for additional case studies from which general principles can be developed. Avian malaria in southern African waterfowl has not previously been studied. As a first step towards documenting and understanding its biogeography, we used PCR and molecular sequencing techniques to analyse 454 blood samples from Afrotropical ducks from 5 different locations (spread around the subregion) for avian malaria. Fifty-five blood samples were positive for one or more genera of haematozoa. The regional infection rate across all sites and sampling periods was 12·1%. Nine individuals carried dual infections containing multiple haematozoa. Fifteen different cytochrome *b* haplotypes among 52 positives (3 samples failed to sequence) and 61 total sequences were found. Eleven haplotypes closely matched *Plasmodium*, whereas 4 were more similar to *Haemoproteus*. Five distinct haematozoan clades were identified. *Haemoproteus* parasites appeared to be more host-specific than *Plasmodium*, which occurred at every sampling location and in every host species examined. There were no significant differences in overall parasite prevalence attributable to either site or species, although *Plasmodium* and *Haemoproteus* occurrences differed by site-species combination and the borderline significance of our test for between-site variation ($P < 0{\cdot}06$) implied that with a larger sample size, differences in parasite prevalence among locations might be detectable.

Key words: malaria, waterfowl, southern Africa, parasite, pathogen, evolution, biogeography, ecology.

INTRODUCTION

Analysis of the causes and consequences of spatial variation in the composition of ecological communities has been an important theme in the development of ecological theory. We can be confident for most vertebrates, for example, that biodiversity at a given location is a consequence of processes at multiple temporal and spatial scales (Gaston, 2000), and that dispersal and movement play critical roles in structuring animal communities (Hanski, 1998). Similarly, the relative importance of species sorting processes within a given area, such as competition, predation or disease, is contingent on resource availability, ecosystem productivity (Loreau *et al*. 2001), the magnitudes of both spatial and temporal variation (Rahbek, 2005), and the degree to which the ecosystem is open or closed (Ewers and Didham, 2006).

For some important kinds of community, however, general principles relating to spatial variation are less evident. One such puzzle piece is that of the parasite community (Lambin *et al*. 2010). There are at least 3 important questions relating to spatial variation in parasite communities to which we do not yet have definitive general answers. First, to what extent is spatial variation in most parasite communities driven by the external environment versus the composition of the vector and/or host communities? Second, how do feedbacks from parasites to their hosts and vectors influence spatial patterns of host biodiversity? And third, given that parasites often co-evolve with their hosts and vectors (Fallon *et al*. 2005), and may evolve more rapidly than either, how do the (usually) long-term dynamics of evolution interact in both space and time with the shorter-term ecological dynamics of host movements and community change?

Although a few research programmes have addressed some or all of these questions for particular systems (Loiseau *et al*. 2012), the field as a whole lacks well-established general principles that explain spatial variation in parasite communities. One of the critical needs in this context is thus for focused case studies that will help researchers to understand the distinctions between true general principles and case-specific detail. For example, the concept of dilution (i.e., the idea that if host competence differs, the

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transmission potential of parasites will be lower in a more diverse animal community) has been well supported for Lyme disease (e.g., Keesing *et al*. 2006), but its more general validity is not supported in many other host-parasite systems (Randolph and Dobson, 2012).

In this paper we present a first analysis of a previously little-explored case study system, that of waterfowl and avian malaria in southern Africa. Waterfowl offer a potentially interesting test case because they spend much of their time in wetlands that are highly suitable for mosquitoes; they are known to move widely throughout the subregion (Hockey, 2000); and most of the Afrotropical waterfowl species move only within the African continent and therefore remain throughout the year in areas in which avian malaria is endemic. The host community exhibits high levels of both spatial and temporal variation and the system thus provides valuable opportunities for examining the relevance of environmental variation and host movement patterns for host-parasite interactions, landscape epidemiology, and parasite biogeography.

All 3 genera of avian malaria haematozoa – *Plasmodium, Haemoproteus* and *Leucocytozoon* – occur in the subregion (Valkiūnas, 2005). *Plasmodium* spp. and *Haemoproteus* spp. were previously believed to have no detrimental effects on the avian host's health, but recent data suggest that infections by these genera can reduce survival, health status, and reproductive performance in the infected host (Marzal *et al*. 2008).

Given the nearly complete lack of background knowledge concerning our study system, we focus in this first analysis on 4 relatively straightforward questions: (1) how common is avian malaria in waterfowl within the southern African region, and does its prevalence vary significantly across the region? (2) Is avian malaria predominantly host specific, location specific, or some combination of the two? (3) Are there seasonal differences in the occurrence of avian malaria, based on expected changes in populations of insect vectors? And (4), are there consistent differences between sites or species in occurrences of the major genera of avian malaria (*Haemoproteus* and *Plasmodium*)? To answer these questions, we examined haematozoa infections in 454 individual birds from 8 waterfowl species (family Anatidae) belonging to 4 genera (*Anas*, *Alopochen*, *Plectropterus*, and *Dendrocygna*) that were sampled at 5 different locations in southern Africa.

MATERIALS AND METHODS

Field sites, study species, and sampling protocols

Blood samples were collected from 454 individual birds from 5 locations (Fig. 1): Strandfontein Wastewater Treatment Works (STR), near Cape Town in the Western Cape Province of South Africa; Barberspan (BAR), near Delareyville in the Northwest Province of South Africa; Lake Manyame and Lake Chivero (ZW), adjacent man-made dams near Harare, Zimbabwe; Lake Chuali (CHU), about 100 km north of Maputo in Mozambique; and Lake Ngami (NGA), the southern end of the Okavango system in Botswana.

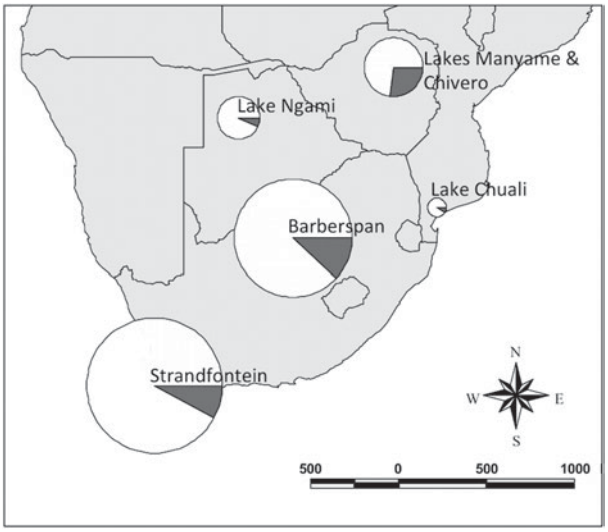

Fig. 1. Locations in southern Africa from which blood samples were collected. In each case, the centre of the pie chart matches the location of the site. Pie size is proportional to the number of samples that were tested; red shading indicates the number of samples that were positive for any haematozoa and white shading indicates negatives. Actual numbers are given in Table 1. Distances on the Scale bar are in kilometres.

The 8 waterfowl (Anatidae) species included in this study were Cape Shoveler (*Anas smithii*), Cape Teal (*Anas capensis*), Hottentot Teal (*Anas hottentota*), Red-billed Teal (*Anas erythrorhyncha*), Yellow-billed Duck (*Anas undulata*), Egyptian Goose (*Alopochen aegyptiaca*), Spur-winged Goose (*Plectropterus gambensis*), and White-faced Whistling Duck (*Dendrocygna viduata*). Birds were captured using walk-in traps, mist nets, and cannon nets. Blood samples were collected by pricking the tarsal vein and capturing a small amount of blood in a capillary tube. Blood was transferred from the tube immediately into lysis buffer and stored in a sealed vial. Sampling was undertaken regularly over the course of 2 full calendar years, with 3 sites (Strandfontein, Barberspan, and Zimbabwe) being sampled every 2 months and 2 sites (Lakes Chuali and Ngami) being sampled every 4 months. Full details of the timing and location of each sampling event have been published by Cumming *et al*. (2011).

Molecular analysis

DNA extraction was carried out in the laboratory using the DNeasy tissue kit protocol (Quiagen).

Following the protocol of Waldenström *et al.* (2002), a nested polymerase chain reaction was used to target a 478 bp fragment of the mitochondrial cytochrome *b* gene from the genera *Haemoproteus* and *Plasmodium*; the primers used do not amplify DNA from *Leucocytozoon* spp. (while we would have liked to include phylogenetic analysis of *Leucocytozoon*, costs were prohibitive). The reaction mix consisted of 8 μl of water, 12·5 μl of GoTaq Green Master Mix (Promega Corporation, Madison, WI, USA), 1·5 μl of primer (10 nM) Haem NF (5′- CATATATTAAGAGAATTATGGAG-3′), 1·5 μl of primer (10 nM) Haem NR2 (5′-AGAGGTGTAGCATATCTATCTAC-3′), and 2 μl of DNA extract for a total reaction volume of 25 μl. The PCR profile was as follows: 94 °C for 3 min, 35 cycles of 94 °C for 30 sec, 50 °C annealing for 30 sec, and 72 °C extension for 45 sec, and finally a 72 °C step for 10 min. A second, nested PCR was then performed with 1·5 μl of the first PCR product and the nested set of primers (10 nM) Haem F (5′- AATGGTGCTTTCGATATATGCATG-3′) and Haem R2 (5′-GCATTATCTGGATGTGATAATGGT-3′), using the same reaction mix and PCR profile as above. We used 1·5% gel electrophoresis (agarose gels) to test for the presence of a PCR product indicative of a positive infection. Positive PCR products were cleaned with 1·8 μl of Agencourt Ampure beads following the manufacturer's protocols (Beckman Coulter, Danvers, MA, USA). PCR products were sequenced using Big Dye v.3.1 dye-terminator chemistry following the manufacturer's protocols (Applied Biosystems, Valencia, CA, USA). Automated sequencing was performed at the DNA sequencing facility on Science Hill at Yale University on an ABI 3730. Two trials were conducted for each sample. Sequences were aligned and edited using Sequencher (Gene Codes Corporation, Ann Arbor, MI, USA).

A Bayesian phylogeny of haematazoa haplotypes was constructed using MrBayes vers. 3.2.1 (Ronquist *et al.* 2012) and a general time reversible (GTR) substitution model with invariant sites (pinvar) and a gamma distribution of among-site rate variation (alpha). For this analysis, we ran 2 000 000 generations, sampling parameters and trees every 1000 generations; we discarded the first 25% of samples (i.e., 500 000 generations) as burn-in. The analysis was replicated which produced similar results and split frequencies were less than 0·01 at the end of each run. We also examined the partitioning of molecular variation among species and among sites using analyses of molecular variance (AMOVA; Excoffier *et al.* 1992) in the program Arlequin 3.1 (Excoffier *et al.* 2005). Species and sites were tested in separate AMOVAs. For each analysis, we excluded categories (species and sites, respectively) that contained fewer than 4 infections.

To examine evidence for the specificity of haematozoan lineages to Anseriformes, available cytochrome b sequence data for southern African birds were downloaded from GenBank. After excluding all accessions that had less than 300 bp of data, we obtained a total of 67 sequences from South Africa ($N = 18$), Botswana ($N = 42$), and Zimbabwe ($N = 7$). Sequences were obtained from Waldenström *et al.* (2002), Ishtiaq *et al.* (2006, 2012), Beadell *et al.* (2006), and Durrant *et al.* (2007). Most sequences were isolated from avian hosts from the order Passeriformes (perching birds; $N = 62$), but also from Coraciiformes ($N = 2$), Columbiformes ($N = 1$), Pelecaniformes ($N = 1$), and Sphenisciformes ($N = 1$), and sequences included both *Haemoproteus* spp. ($N = 38$) and *Plasmodium* spp. ($N = 29$). Including the 61 sequences obtained from Anseriformes for a total of 128 sequences, we constructed a Bayesian phylogeny using MrBayes vers. 3.2.1 as described above, except that we ran a total of 3 000 000 generations.

Statistical analysis

Impressions of prevalence and host specificity can be strongly influenced by sampling biases. Despite virtually constant sampling effort, our samples did not include a representative or standardized sample of the birds present at each site, for the simple reason that some species in some locations were extremely difficult to catch. Similarly, although we sampled year-round over a 2-year period, abundances of birds varied considerably and for most species there was a single time of year (often corresponding to the period before, during, or after flightless moult) when individuals were most abundant and easiest to catch at our study sites. To further complicate the analysis, the processes driving duck abundance are not synchronized across the region, with substantial variation occurring in the timing of rainfall and periods of high and low water. Notable differences in hydrology occur not only between summer and winter rainfall sites but also as a consequence of the 'delayed' flow regime of Lake Ngami (which receives most of its water from the Angolan highlands, via the lower end of the Okavango Delta). Although we started with simple calculations of overall prevalence across all species, more rigorous analysis and interpretation of the data had to be undertaken to correct for biases deriving from differences in location, species composition, and sampling time. All analyses were run in Matlab R2010b (Mathworks, 2010).

The methods for each of our focal questions are now considered in turn. The first was how common is avian malaria in waterfowl within the southern African region and whether its prevalence varies significantly across the region. To address this question we quantified both overall regional prevalence (i.e., pooling all samples into a simple overall statistic) and prevalence by site-species combination (i.e., prevalence within each species at each site). We tested for significant variation in prevalence among our study sites using an ANOVA by site of the site-species prevalence data, ignoring seasonal differences.

Our second question was that of whether there were seasonal differences in the occurrence of avian malaria in waterfowl. To test for a seasonal effect while correcting for sampling biases, we used a Mann-Whitney U-test (given that a Lilliefors test of the site-species-month prevalence data indicated significant departure from normality, $P<0{\cdot}001$) to compare the prevalence for each 'actual' species-site-month combination against a null hypothesis that assumed that the number of positives would be a consistent proportion of the total number of birds of a given species sampled at that site in each month. This analysis used only data for bird species that were sampled in a given month at a given site. Because of sample size constraints, we ran the analysis using data from all duck species and including all positives (i.e., data from both *Plasmodium* spp. and *Haemoproteus* spp.). The 28 rows of data for which both observed and expected values were 0 were removed from this analysis to avoid over-estimation of degrees of freedom (and hence, biasing the results towards significance), leaving a sample size of 58 observations for this test. We did not have a sufficiently large sample size to search for seasonal trends in data from individual sites.

Our third question was that of whether avian malaria is predominantly host specific, location specific, or some significant combination of the two. The challenge in addressing this question is again to correct for sampling bias. It can be re-phrased as the question of whether variation in malaria prevalence can be mainly ascribed to species influences, site influences, or a combination factor of the two. Given the (insignificant) outcome of the analysis for seasonal differences in malaria prevalence, we were justified in ignoring seasonality and comparing species and site effects using an N-way ANOVA (implemented through the Matlab procedure *anovan*) with prevalence as the response measurement and site and species as factors. We used the N-way ANOVA in preference to the two-way ANOVA because it is more robust to missing data, which were common in our data set because of differences in bird captures between sites (unsampled species cannot simply be assigned a prevalence of zero).

Our fourth question was that of whether there are consistent differences between sites or species in occurrences of the 2 major genera of avian malaria (*Haemoproteus* and *Plasmodium*). We tested for differences using data for all birds that tested positive for either parasite, first running a Mann-Whitney U-test to determine whether parasite occurrences for all unique site-species combinations differed and then an N-way ANOVA to test whether either site or species, as factors, explained a significant proportion of variance in the numbers of either parasite.

RESULTS

In the first PCR trial, using 454 individual birds, positive infections of haematozoa were detected in 46 (10·1%) individuals (Table 1). In the second trial, positives were detected in 34 (7·5%) individuals. Twenty-six individuals were positive in both trials, whereas 29 positives were either negative or ambiguous in one of the trials. Overall, 55 samples were positives in at least one trial. Nine individuals that were positive for haematozoa showed polymorphisms in their sequences. These samples were sequenced again with new PCR product to test for the possibility of contamination in the first trial. Upon verification that there were multiple peaks in the sequences, they were labelled as dual infections, containing multiple haematozoa. Two sequences were then designated for each of the individuals by comparing nucleotide polymorphisms between the ambiguous sequence and all resolved haplotypes. In each case, we were able to identify 2 haplotypes that, when combined, resulted in the same polymorphic sites as observed in the ambiguous sequence.

Fifteen different cytochrome *b* haplotypes were found among 52 positives (3 samples failed to sequence) and 61 total sequences (Fig. 2). GenBank BLAST searches revealed that 11 haplotypes closely matched *Plasmodium* spp., whereas 4 haplotypes were more similar to *Haemoproteus*. Of the 9 individuals carrying dual infections, 5 were infected by 2 lineages of *Plasmodium*, 3 were infected by 2 lineages of *Haemoproteus*, and 1 was infected by both *Plasmodium* and *Haemoproteus*. Based on posterior support of $\geqslant 0{\cdot}95$, five distinct clades (A–E) can be identified (Fig. 2), although 3 individuals with unique haplotypes did not fit into these clades. Sixteen *Haemoproteus* spp. parasites were detected and sequenced, revealing 4 different haplotypes. Ignoring sampling bias for the moment, *Haemoproteus* spp. infections appeared to be most common in Egyptian Geese ($n=11$; 68·8% of *Haemoproteus* spp. infections), but were also found in Cape Teal ($n=2$; 12·5%), Yellow-Billed Duck ($n=2$; 12·5%), and Red-Billed Teal ($n=1$; 6·3%). Fifteen of the 16 *Haemoproteus* spp. parasites were isolated from sampling localities in South Africa (BAR and STR) and the remaining one from the site in Bostwana (Figs. 2 and 3). No *Haemoproteus* spp. were detected in the Zimbabwean and Mozambican sites, the 2 most eastern sites. With 45 sequences, *Plasmodium* spp. showed a wide range of infection hosts and locations, occurring at every sampling location and in every host species examined. Although lineages C and D were most prevalent in Yellow-billed Ducks, lineages A and B were both found in a wide variety of hosts. Spur-winged geese from Zimbabwe showed a particularly high infection by *Plasmodium* spp., with infections being detected in 6 of the 7 individuals sampled (Fig. 3), 3 of which

Table 1. Results, by site and species, giving the number of birds sampled of each species and the number that were positive for the genera *Plasmodium* and *Haemoproteus*

(Blank entries indicate zero prevalence rather than missing data. For clarity, the dual and unidentified infections were excluded from this table. Site names are STR, Strandfontein; BAR, Barberspan; MAN, Lake Manyame; CHI, Lake Chivero (MAN and CHI were merged into ZIM for the analysis); NGA, Lake Ngami; CHU, Lake Chuali.)

Site and Species	Birds Sampled	Positive for *Plasmodium*	Positive for *Haemoproteus*
BAR	148	12	4
Cape Shoveler	17		
Cape Teal	18		
Egyptian Goose	35	1	4
Red-billed Teal	22	4	
White-faced Duck	6		
Yellow-billed Duck	50	7	
CHI	20	6	
Egyptian Goose	5	1	
Spur-winged Goose	7	3	
White-faced Duck	8	2	
CHU	23	2	
Hottentot Teal	4		
Red-billed Teal	5		
White-faced Duck	10	2	
Yellow-billed Duck	4		
MAN	41	7	
Cape Shoveler	1	1	
Egyptian Goose	6		
Hottentot Teal	10	2	
Red-billed Teal	22	3	
White-faced Duck	2	1	
NGA	44	2	1
Egyptian Goose	4		
Hottentot Teal	13		
Red-billed Teal	21	2	1
White-faced Duck	6		
STR	178	5	5
Cape Shoveler	6		
Cape Teal	17	2	3
Egyptian Goose	103	1	2
Red-billed Teal	25	1	
Spur-winged Goose	10		
Yellow-billed Duck	17	1	
Total	454	34	10

contained multiple lineages (Fig. 2). Interestingly, all positive infections from the north-eastern sites (Zimbabwe, $N=21$ infections; Lake Chuali, $N=2$ infections) were *Plasmodium* lineages A and B, compared to 23·6% (9 of 38 infections) elsewhere in southern Africa.

The regional infection rate across all sites and sampling periods was 12·1%. When considered without corrections for sampling biases, regional infection rates varied significantly among host species ($\chi^2=19{\cdot}6$, D.F.$=7$, $P<0{\cdot}01$) and sampling locations ($\chi^2=17{\cdot}62$, D.F.$=4$, $P<0{\cdot}001$) (Fig. 3). In addition, 37% of the total molecular variation was partitioned among the 6 species that had $\geqslant 4$ infections (AMOVA, $P<0{\cdot}00001$), and 38% was partitioned among the 3 sites that had $\geqslant 4$ infections ($P<0{\cdot}00001$). Similar values were obtained when confining analyses to *Plasmodium* lineages only (32% among 5 species and 36% among 3 sites; $P<0{\cdot}00001$ in both cases).

The mean avian malaria prevalence value calculated for all site-species combinations was 16%. This value was heavily influenced by the fact that the single sampled Cape Shoveler from Zimbabwe was positive, giving 100% prevalence for this site-species combination. With Cape Shoveler in Zimbabwe removed, site-species mean prevalence was 12·6%. Haematozoan prevalence across all unique site-species-month combinations did not differ significantly from a null data set that assumed an even distribution across species within a site by month (Mann-Whitney U-test, $n=58$, $P<0{\cdot}14$), suggesting that there was no regional (overall) seasonal trend in malaria infections, although this test does not correct for regional differences in rainfall.

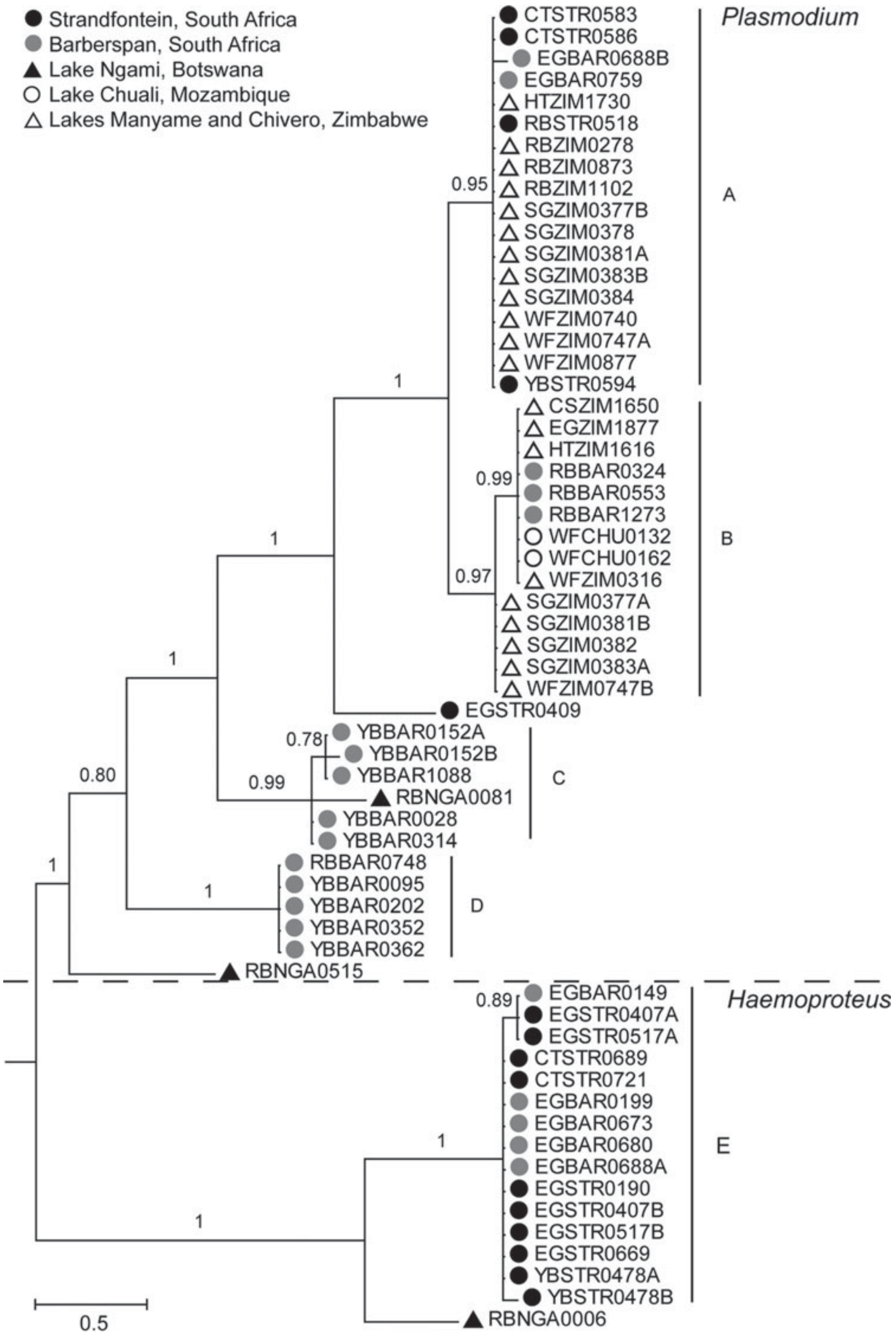

Fig. 2. Bayesian phylogenetic tree of mtDNA cytochrome *b* haplotypes obtained from haematozoa sample from waterfowl (pinvar = 0·46, alpha = 0·091). Sample names ending in an A or B indicate individuals that were infected by multiple haplotypes. Letters next to each vertical line indicate specific mtDNA lineages supported by >95% bootstrap support and that are referenced in the text. The horizontal dashed line separates *Plasmodium* and *Haemoproteus* haplotypes. The first 2 letters in each label indicate host species; Cape Shoveler (CS), Cape Teal (CT), Red-billed Teal (RT), Hottentot Teal (HT), Egyptian Goose (EG), Spur-winged Goose (SW), and White-faced Whistling Duck (WF). Numbers above branches indicate posterior nodal support.

An N-way ANOVA indicated that there were no significant differences in prevalence attributable to either site or species ($n = 26$; $F = 0{\cdot}79$, $P < 0{\cdot}6$ for species; $F = 2{\cdot}92$, $P < 0{\cdot}06$ for site). Unfortunately our data set was not large enough to quantify interaction effects between factors. The greater F value for site as a factor, and its borderline significance, suggest that a larger sample size might support the argument that differences in parasite levels between locations are greater than the differences between host species.

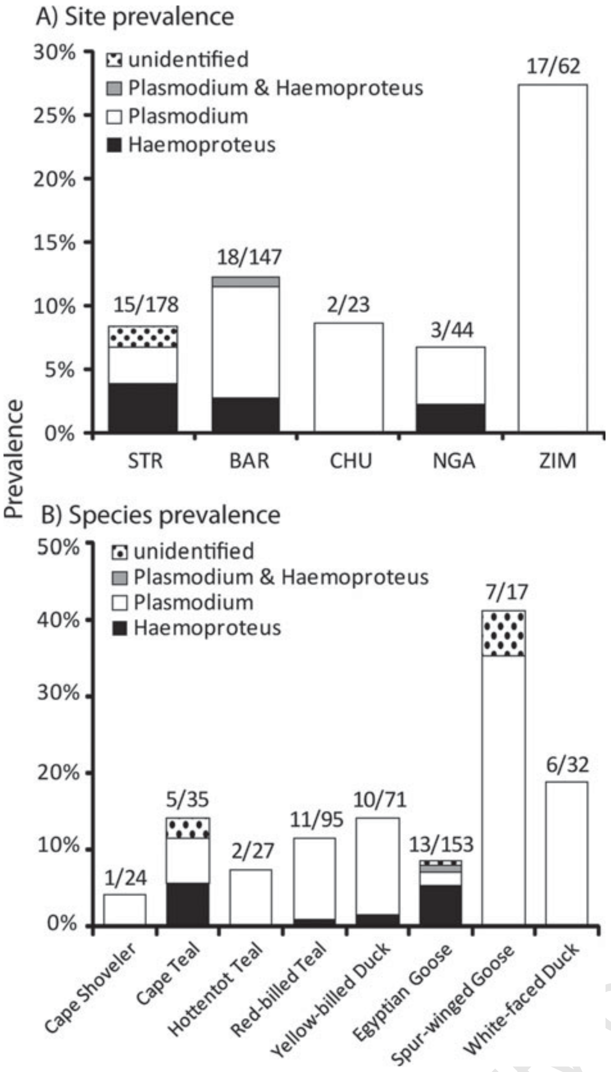

Fig. 3. Haematozoa infections by (A) location and (B) host species. The numbers above each column indicate the number of positive samples and the number that were tested.

Analysis of differences between infections for the two different haematozoa genera, *Plasmodium* and *Haemoproteus*, indicated significant differences in parasite occurrences by site-species pairs (Mann-Whitney U-test, $P < 0{\cdot}0015$, $n = 16$). However, tests of each individual data set for differences by site and species did not indicate a significant explanatory effect of either factor (N-way ANOVA, $n = 16$; for *Plasmodium* spp., for species and site respectively, $F = 0{\cdot}86$ and $0{\cdot}79$; $P < 0{\cdot}6$ in both cases; for *Haemoproteus* spp., for species and site respectively, $F = 2{\cdot}08$ and $1{\cdot}42$; $P < 0{\cdot}29$ and $0{\cdot}41$).

Comparing haematozoan lineages isolated from Anseriformes with those isolated from other birds from southern Africa, we identified 2 major groups (1 *Haemoproteus* spp. and 1 *Plasmodium* spp.) and 1 minor group (*Plasmodium* spp.) that were exclusive to Anseriformes (Fig. 4). These monophyletic groups collectively included 4 of the lineages ('A', 'B', 'D' and 'E') identified in Fig. 2, as well as 2 of the 3 unique haplotypes that did not fit into any lineage. Lineage 'C', however, was present in both Anseriformes and Passeriformes and included a haplotype that was shared between the two orders. The *Plasmodium* sp. isolate from RBNGA0515 (Red-billed Teal, *Anas erythrorhyncha*) did not group with other Anseriformes lineages, but rather grouped with isolates from Passeriformes, Pelecaniformes, and Coraciiformes with high posterior probability (0·99; Fig. 4). With the exception of lineage 'C', all Anseriformes isolates were well differentiated from haematozoa from other orders. Furthermore, 1 isolate from Pelecaniformes had an identical sequence to isolates from several Passeriformes, whereas haematozoa obtained from Coraciiformes, Columbiformes, and Sphenisciformes were unique and well differentiated from other isolates.

DISCUSSION

Given that avian malaria prevalence in southern African waterfowl has not previously been documented, our results show some interesting patterns. Avian malaria occurs in waterfowl at an average prevalence of around 12% across southern Africa. Infection with *Plasmodium* spp. appears to be approximately 3 times more common than infection with *Haemoproteus* spp. Infections in waterfowl in our study seem to have a non-random distribution with respect to the mtDNA lineage of haematozoa. Interestingly, waterfowl haematozoa were genetically differentiated from haematozoa infecting other avian orders, except for 1 haematozoan lineage that was shared between waterfowl and perching birds.

Plasmodium spp. infected a wide range of waterfowl from different wetlands, with some strains (particularly lineage 'A' in Fig. 2) appearing to be both generalist and ubiquitous and other lineages (e.g., 'D' in Fig. 2) appearing to be more localized and species specific. Larger sample sizes will be required to adequately determine levels of host specialization by haematozoan species, but our initial results suggest that different species of *Plasmodium* may cover the spectrum from specialist to generalist (Beadell *et al*. 2009; Hellgren *et al*. 2009). *Haemoproteus* spp. appeared subjectively to show greater host specificity than *Plasmodium* spp. Studies in West Africa and elsewhere (Beadell *et al*. 2004, 2009; Bensch *et al*. 2009) have found that *Haemoproteus* spp. generally show more host specificity than *Plasmodium* spp. Beadell *et al*. (2004) found compelling evidence that *Plasmodium* spp. can parasitize multiple host families while *Haemoproteus* spp. species tend to be more specialized. However, our results indicate that some *Plasmodium* spp. specialize on particular hosts within particular avian orders. For example, although lineages A and B infected a wide range of waterfowl hosts, these species have not been found in other avian orders.

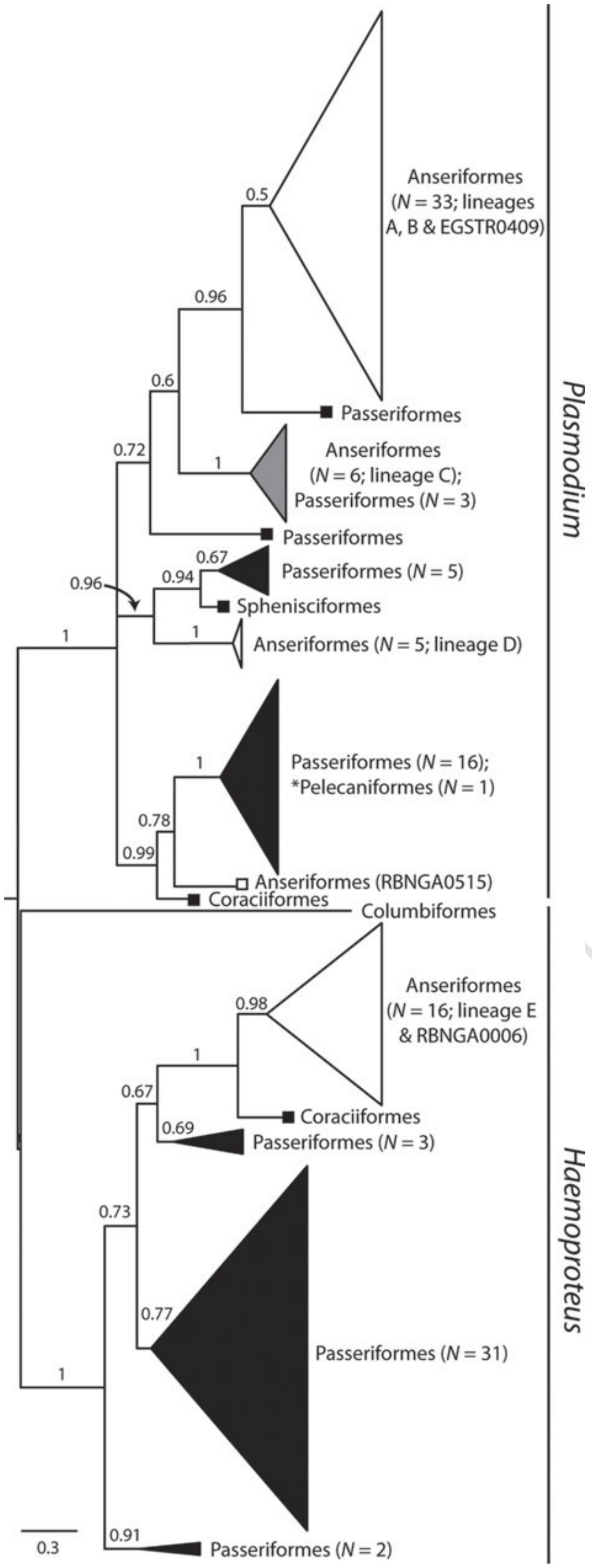

Fig. 4. Bayesian phylogeny of 128 mtDNA cytochrome *b* haplotypes isolated from southern African birds (pinvar = 0·29, alpha = 0·11). Monophyletic clades for Anseriformes isolates (white triangles) and Passeriformes (black triangles) were collapsed for clarity (the asterisk (*) indicates one Passeriformes clade that included a haplotype shared with a single isolate from Pelecaniformes). The triangles illustrate the abundance (height) of the clade and the deepest divergence (width) within the clade. One clade (grey triangle) contained *Plasmodium* spp. isolated from both Anseriformes and Passeriformes, including a haplotype that was shared between these orders. Numbers above branches indicate posterior nodal support.

A recent study on passerines in the western Cape (Schultz *et al*. 2011) suggested a higher overall prevalence and a higher diversity of avian haemosporidia in passerines than found in waterfowl in this study. Schultz *et al*. (2011), however, based species identification on visual identification from gametocyte morphology and their results are thus difficult to compare directly to ours. Their samples were dominated by *Leucocytozoon* spp., with *Plasmodium* spp. infections detected in only 3·2% of sampled birds; we found a much higher incidence of *Plasmodium* spp.

The lack of a seasonal trend within our data was surprising, given that outside the winter rainfall region we would expect to find strong declines in mosquito and culicoides (biting midge) abundance during the drier and colder periods of the year (Altizer *et al*. 2006). Other studies on avian malaria have also found a similar lack of a seasonal or annual trend in infection prevalence (e.g., Bensch *et al*. 2007, although this study misses some annual variation), which invites speculation as to the extent of the influence of vector population dynamics. Although no data on vector population abundance were collected for this study, it would be interesting to determine whether their inclusion would explain variation in avian malaria prevalence. It is possible that, as with avian influenza, a combination of long-distance movement by host species and high levels of variation in infection rates swamp any seasonal influences (Cumming *et al*. 2011). Alternatively, although temperature and photoperiod vary synchronously across the region (with a routinely cold June/July and warm December/January), temperature differences are not always mirrored by differences in rainfall. Given that malaria prevalence may be driven by the covariance between temperature and rainfall (Hay *et al*. 2002), a more sophisticated climate matching procedure to fully eliminate the hypothesis of a rainfall-driven ‘malaria season’ is needed. Also of relevance on this theme is that little is known about latency and the expression of malaria in waterbirds; it is possible that hyponozoites may remain in the liver for long periods as they do in humans (e.g., White, 2011), confusing our understanding of seasonal transmission dynamics.

Taken together, our results suggest that haematozoa species in southern Africa tend towards being generalist but localized parasites of waterfowl, rather than ubiquitous host specialists. More extensive sampling of our focal species will be needed before we can determine whether any particular strains of haematozoa are linked conclusively to particular locations or regions. Our initial results are nonetheless promising, in that they imply that analysis of the biogeography and host use of avian malaria may provide a workable model system for understanding how spatial variation in waterfowl community composition and movement patterns influences their susceptibility to parasitic infections.

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ECOHEALTH

Original Contribution

The Ecology of Influenza A Viruses in Wild Birds in Southern Africa

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Abstract: Avian influenza viruses (AIVs) are pathogens of global concern, but there has been little previous research on avian influenza in southern Africa and almost nothing is known about the dynamics of AIVs in the region. We counted, captured and sampled birds regularly at five sites, two in South Africa (Barberspan and Strandfontein) and one in each of Botswana (Lake Ngami), Mozambique (Lake Chuali) and Zimbabwe (Lakes Manyame and Chivero) between March 2007 and May 2009. The South African and Zimbabwean sites were visited every 2 months and the sites in Botswana and Mozambique every 4 months. During each visit we undertook 5–7 days of standardised bird counts followed by 5–10 days of capturing and sampling water-associated birds. We sampled 4,977 birds of 165 different species and completed 2,503 half-hour point counts. We found 125 positive rRT-PCR cases of avian influenza across all sites. Two viruses (H1N8 and H3N8) were isolated and additional H5, H6 and H7 strains were identified. We did not positively identify any highly pathogenic H5N1. Overall viral prevalence (2.51%) was similar to the lower range of European values, considerable spatial and temporal variation occurred in viral prevalence, and there was no detectable influence of the annual influx of Palearctic migrants. Although waterbirds appear to be the primary viral carriers, passerines may link wild birds and poultry. While influenza cycles are probably driven by the bird movements that result from rainfall patterns, the epidemiology of avian influenza in wild birds in the subregion is complex and there appears to be the possibility for viral transmission throughout the year.

Electronic supplementary material: The online version of this article (doi:10.1007/s10393-011-0684-z) contains supplementary material, which is available to authorized users.

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INTRODUCTION

Influenza A viruses have long been acknowledged as pathogens of global concern. In recent years, outbreaks of highly pathogenic avian influenza (HPAI) in populations of domestic and wild birds, and the related deaths of nearly 300 people (WHO, 2010), have heightened fears of a new influenza pandemic in the human population (e.g. Pickles, 2006; Enserink, 2006). Assessments of the risks that are posed by avian influenza, and the development of appropriate response strategies in the event of an epidemic or pandemic, rely heavily on a fundamental scientific understanding of avian influenza virus (AIV) dynamics in populations of domestic and wild birds (Dudley, 2008).

Although low pathogenic avian influenza (LPAI) viral prevalence in western European and North American wild bird populations has been well documented (Olsen et al., 2006), it is unclear how the long-distance movements of migratory and nomadic bird species relate to larger-scale spatial and temporal variation in AIV genotypes, maintenance, and epizootics/epidemics (Krauss & Webster, 2010; Kilpatrick et al., 2006). One of the largest single gaps in the geographical coverage of AIV sampling has been southern Africa (Olsen et al., 2006; Kilpatrick et al., 2006; Gaidet et al., 2007), a region that is at risk following the detection of highly pathogenic strains in sub-Saharan Africa north of the Zambezi (Gaidet et al., 2008; Fasina et al., 2009). Although some intriguing data exist from South Africa (such as the finding that precursors to pathogenic AIV strains are introduced to and possibly moved between ostrich farms by Egyptian Geese *Alopochen aegyptiacus*; e.g. see Abolnik et al., 2010; Abolnik et al., 2009; Sinclair et al., 2005), little relevant research has been carried out in most southern African countries.

By comparison to western Europe, southern Africa has a mild winter; highly variable and often scarce rainfall; a higher diversity of bird species; no true geese or swans; and many nomadic waterbirds but no truly migratory afrotropical *Anas* ducks (Cumming et al., 2008; Underhill et al., 1999). We tested the predictions that (1) due to its more arid environment and absence of migratory Palearctic ducks, LPAI prevalence in wild waterbirds should be lower in southern Africa than in Europe; (2) due to the presence of many opportunistic, colonial and nomadic waterbird species, and the lack of migratory corridors (Hockey, 2000), LPAI prevalence in wild birds in southern Africa should show relatively little spatial variation along longitudinal and latitudinal gradients; and (3) the arrival of Palearctic migrants in September (see Appendix 1 in Supporting information for details), including charadriids known as potential LPAI reservoirs, should create a pulse in influenza occurrences in Afrotropical species.

While exploring these fundamental assumptions for the first time, we also provide a wealth of new and useful information on AIV and wild birds in southern Africa. Our results suggest that none of our starting assumptions can be strongly supported. Some re-thinking of prevailing assumptions about influenza A viruses in southern African bird populations thus appears necessary in planning health care and risk management strategies.

METHODS

Project Design and Field Sites

Data were collected in Botswana, Mozambique, South Africa and Zimbabwe from March 2007 to May 2009. We worked on a regular basis at five different sites (Fig. 1) and 12–15 sampling locations per site. We counted and sampled birds at daily, bimonthly and annual time scales. Our three core sites [Barberspan and Strandfontein in South Africa, and the Manyame catchment in Zimbabwe (including Lakes Chivero and Manyame)] were sampled every 2 months and our Botswana site (Lake Ngami) and Mozambique site (Lake Chuali) every 4 months. We also sampled a small number of birds during a single 'test' sampling mission to Massingir Dam in Mozambique. Exact sampling dates and coordinates of capture sites are given in Appendix 2 (Supporting information) and additional details on study sites in Appendix 3 (Supporting information).

Counting Protocols

Each site visit included 5–7 days of standardised bird counts followed by 5–10 days of bird captures in the same locations (Fig. 2). Counts consisted of a 10-min habituation period followed by a 30-min counting period, during

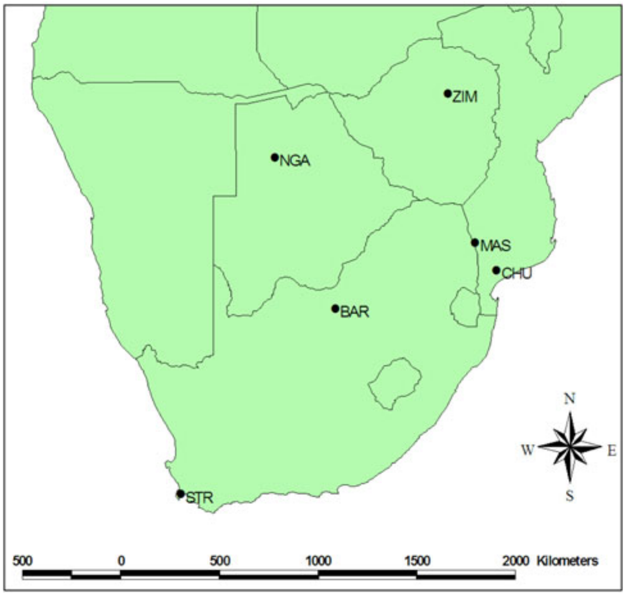

Figure 1. Map of southern Africa showing sampling sites mentioned in this article. Site codes: *ZIM* Lakes Chivero and Manyame, *NGA* Lake Ngami, *MAS* Massingir Dam, *CHU* Lake Chuali, *BAR* Barberspan, *STR* Strandfontein. Our three core sites were STR, BAR and ZIM, which fall in different biomes along a north–south latitudinal gradient.

which the number and species of all birds within a 150 m radius of the (stationary) observers were recorded. Each location was counted at four different times of day over a 5–7 day period prior to captures [additional details in Appendix 3 (Supporting information)]. Over the 2 years of the study we completed 2,503 half-hour point counts. For each of our three core sites (Barberspan, Manyame/Chivero and Strandfontein) the count data also provide estimates from 13 different points in time (i.e. every 2 months for 2 years), giving us a spatiotemporally balanced sampling design for exploring both spatial and temporal variation in the bird community.

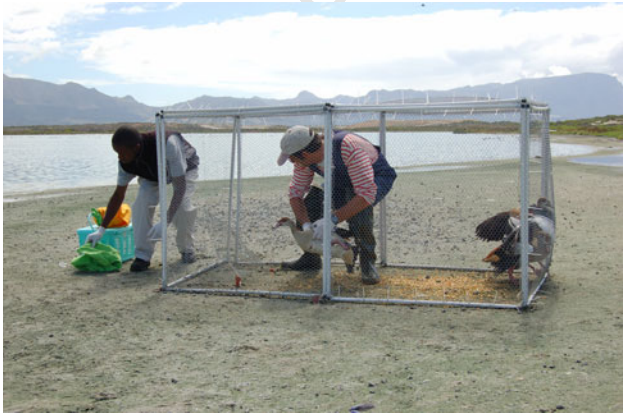

Figure 2. Example of a walk-in trap used to catch ducks. In this picture, Mduduzi Ndlovu (L) and Leo Bruinzeel (R) capture Egyptian geese at Strandfontein.

Capture and Sampling Protocols

Captures used standard procedures as detailed in Appendix 3 (Supporting information). We targeted ducks because they are considered reservoir hosts of some type A AIVs in Europe and Asia. The other sampled species were by-catch (i.e. they were captured during the process of catching ducks). In addition to ancillary data (morphometry, photographs, blood, feathers) we collected two cloacal and two tracheal swabs per bird. Birds recaptured in the same week were not resampled. All swabs were placed in cryovials in viral transport medium (Hank's salt solution with antibiotics and fungicides) and frozen in liquid nitrogen within half an hour of collection.

The swabs were stored in a −70°C freezer and transported in dry ice or liquid nitrogen to an FAO reference laboratory, either the Agricultural Research Council-Onderstepoort Veterinary Institute, Pretoria, South Africa (ARC-OVI) or the Istituto Zooprofilattico Sperimentale delle Venezie, Padova, Italy (IZSVe) for analysis (see Appendix 3 in Supporting information for details). Sets of swabs were randomised by laboratory; each received the first cloacal and second tracheal swab from one bird and the second cloacal and first tracheal swab from the next bird. All samples from Botswana and Mozambique were analysed at IZSVe.

Sources of error included (1) failure to obtain a full complement of swabs, due to bird escapes or shortages of vials; (2) labelling errors; (3) loss or destruction of vials in transit and (4) mistakes in allocation of vials to laboratories. Most of these errors were random and hence unbiased. We had fewer than four swabs per bird in just under 4% of cases. Samples were only sent to IZSVe on completion of the project, giving a delay between sampling and analysis of 2–24 months that may have affected the probability of AIV detection (Forster et al., 2008).

Data Analysis

Virus prevalence was too low to determine the influence of the number of swabs on virus detection probability. Since missing swabs were <4% and randomly distributed by species, we assumed that each sampled bird (rather than each swab) had an equal chance of viral detection. Virus prevalence was calculated as the ratio of the number of influenza viruses detected to the number of birds sampled. Since recaptures were not re-sampled during the same capture mission, and since each sampling effort was at least 2 months apart, we treated samples from recaptures (including birds that we had ringed and those ringed by others) as independent.

Having quantified virus prevalence for each species by site, we calculated overall prevalence for all bird species and all sites. Bird count summaries by site used the average number of birds counted across all point counts.

For the Palearctic migrant analysis we included all birds found in our study sites that were both listed in class 6 (i.e. intercontinental and marine migrants) of the Roberts' database (Hockey et al., 2004) and associated with wetland and estuarine habitats. A full list of Palearctic migrant species and their abundances is presented in the Supporting information in Appendix 1. The total number of foraging and non-foraging Palearctic migrants for each sampling mission was converted to a mean abundance by dividing the total count for a single mission by four, since each point count location was counted four times. Since the total numbers of birds are more relevant than their relative abundance to the role of Palearctic migrants in influenza transmission, we did not divide these data by the number of locations per site. We then used Spearman's rank-order correlations to test for a significant relationship between the number of Palearctic migrants, the abundance of anatids, and virus prevalence.

RESULTS

We sampled a total of 4,977 birds of 165 different species, including 158 recaptures. Captures were distributed unevenly across sites (Table 1) despite comparable sampling effort, with the Zimbabwean site yielding the most birds (n = 1916), followed by Barberspan (n = 1418) and Strandfontein (n = 888). Differences in the composition of species caught resulted primarily from differences in local species composition and catchability. A full listing of the number of individuals of the 165 sampled species is provided in Appendix 1 (Supporting information). Some of the data from Zimbabwe have been presented previously by Caron et al. (2010a).

From 4,977 sampled birds, 125 were influenza A positive, giving a prevalence across all species and sites of 2.51%. The probability of an influenza-positive sample being from a cloacal or a tracheal swab was almost identical (n = 125, P = 0.48 vs. P = 0.52 for cloacal and tracheal swabs, respectively; 5 birds were positive on both cloacal and tracheal swabs, one on both tracheal swabs, and none on both cloacal swabs). Infuenza A virus prevalence across different bird families was uneven (Table 1), with four families (Anatidae, Jacanidae, Charadriidae and Dendrocygnidae) together contributing 72.8% of positive samples; the same four families represented 67.5% of birds captured.

Reliable conclusions cannot be drawn from small sample sizes. We sampled over 20 individuals (i.e. the influence of an outlier was 5% or less) for 18 different bird families. From these families the highest mean prevalence values across all sites occurred in the Alaudidae (larks; 24 birds, 3 positives, prevalence 12.5%) and the Dendrocygnidae (whistling ducks; 234 birds, 12 positives, prevalence 5.15%). Also of note were the Scolopacidae (sandpipers and snipes, 180 birds, 6 positives, prevalence 3.33%), Jacanidae (jacanas, 492 birds, 15 positives, prevalence = 3.05%), Ploceidae (weavers, 165 birds, 5 positives, prevalence = 3.03%), Charadriidae (plovers and lapwings; 458 birds, 12 positives, prevalence = 2.62%) and Anatidae (ducks; 2168 birds, 52 positives, prevalence = 2.4%). Conversely, despite reasonably large sample sizes, no AIV RNA was found in the Columbidae (pigeons and doves; n = 122), Glareolidae (pratincoles and coursers; n = 116) or Ardeidae (herons, egrets and bitterns; n = 88).

There was no spatial synchrony in influenza occurrences, with the prevalence of influenza viruses in any 2-month sampling period not being significantly correlated between any pair of sites (n = 12 or 13, Spearman's rho < 0.43, P not significant to the 0.05 or 0.1 levels in all cases).

Two influenza viruses were isolated and several different strains identified (Table 2). An H1N8 influenza virus was isolated from an Egyptian Goose *Alopochen aegyptiacus* caught at Barberspan (see Abolnik et al., 2010) and an H3N8 influenza virus from a Red-billed Teal *Anas erythrorhyncha* caught at Strandfontein. Type-related information was obtained via rRT-PCR for an additional 22 viruses, which included 10 H5-positive and 10 H7-positive samples as well as two H6-positives. Amplicons from the reactions were insufficient for obtaining DNA sequences, and thus the amino acid sequence at the HA0 cleavage sites could not be determined; it is therefore unknown whether the H5 and H7 viruses were of high or low pathogenicity. H7 strains were only identified from Zimbabwe but were found in five different species.

Table 1. Numbers of Birds Sampled for Avian Influenza, by Family and by Site, and Prevalence of Avian Influenza

Family	BAR	CHU	MAS	NGA	STR	ZIM	Totals	Total prevalence %
Accipitridae	0	0	0	0	2	1	3	0
Alaudidae	6	0	0	1	0	17(3)	24(3)	12.5
Alcedinidae	0	3	1	0	0	9(1)	13(1)	7.7
Anatidae	696(8)	27	0	69(1)	680(8)	698(35)	2170(52)	2.4
Apodidae	1	0	0	0	0	0	1	0
Ardeidae	6	14	0	3	27	35	85	0
Burhinidae	1	1	0	0	0	1	3	0
Caprimulgidae	0	0	1	1	0	3	5	0
Cerylidae	1	10	0	4	0	25(1)	40(1)	2.5
Charadriidae	99	31	10	79	17	225(12)	461(12)	2.6
Ciconiidae	0	0	0	1	0	0	1	0
Cisticolidae	0	0	0	0	0	5	5	0
Columbidae	4	0	0	48	26	44	122	0
Coraciidae	0	0	0	0	0	4	4	0
Dacelonidae	0	0	0	0	0	8	8	0
Dendrocygnidae	5	13	1	9(2)	0	206(10)	234(12)	5.1
Estrildidae	0	0	0	2	0	5	7	0
Fringillidae	0	0	0	0	0	1	1	0
Glareolidae	0	13	3	79	0	21	116	0
Haematopodidae	0	0	0	0	4	0	4	0
Hirundinidae	0	2	0	1	3	7(1)	13(1)	7.7
Indicatoridae	0	0	0	0	0	1	1	0
Jacanidae	1	116	0	39	0	337(15)	493(15)	3
Laniidae	1	0	0	0	0	2	3	0
Laridae	3	0	0	2	42	16	63	0
Lybiidae	1	0	0	0	0	2	3	0
Malaconotidae	0	0	0	0	0	2	2	0
Meropidae	0	0	0	1	0	0	1	0
Motacillidae	2	1	0	0	10	30(2)	43(2)	4.7
Muscicapidae	1	0	0	1	1	1	4	0
Numididae	10(1)	0	0	0	8	5	23(1)	4.3
Passeridae	2(1)	0	0	1	3	2	8(1)	12.5
Phalacrocoracidae	0	1	0	0	5	2	8	0
Phasianidae	2	0	0	8	7	3	20	0
Phoenicopteridae	7	0	0	0	0	0	7	0
Ploceidae	25	17	0	32	10	81(5)	165(5)	3
Podicipedidae	0	1	0	0	0	1	2	0
Pycnonotidae	2(1)	0	0	0	3	3	8(1)	12.5
Rallidae	491(7)	2	0	1	13	7	514(7)	1.4
Recurvirostridae	4	0	0	4	8	0	16	0
Rostratulidae	1	6	0	25	0	0	32	0
Scolopacidae	36	11	0	48	0	86(7)	181(7)	3.9
Sturnidae	0	0	1	4	2	9	16	0
Sylviidae	3	2	0	1	0	7(2)	13(2)	15.4
Threskiornithidae	1	0	0	3	15(1)	1	20(1)	5
Tytonidae	4	0	0	0	1	2	7	0
Upupidae	2(1)	0	0	0	0	1	3(1)	33.3

Table 1. continued

Family	BAR	CHU	MAS	NGA	STR	ZIM	Totals	Total prevalence %
Zosteropidae	0	0	0	0	1	0	1	0
TOTALS	1418(19)	271	17	467(3)	888(9)	1916(94)	4977(125)	0

Numbers in brackets indicate the number of birds that tested positive for avian influenza (these are included only when $n > 0$). BAR, Barberspan; CHU, Chuali; MAS, Massingir; NGA, Ngami; STR, Strandfontein; ZIM, Zimbabwe (Chivero and Manyame). Sample sizes for Anatidae were similar across our three core sites (BAR, ZIM, STR). Some of the most obvious differences in bird species composition between sites occurred in the Jacanidae (jacanas; mostly ZIM and CHU), Dendrocygnidae (whistling ducks; mostly ZIM) and Rallidae (coots and rails; mostly BAR).

Influenza viruses are in circulation across the subregion throughout the year (Fig. 3), with no obvious pattern in relation to temperature or rainfall. Patterns between years also appear to be inconsistent, with peaks in viral prevalence in December 2007 and January 2008 in Zimbabwe and Barberspan not present in 2008–2009.

These data should be interpreted within the context of the sampled bird communities. We had relatively high numbers of influenza-positive birds from each of four avian families: Anatidae, Charadriidae, Dendrocygnidae and Jacanidae. The birds in each of these families show differing seasonal trends in abundance as well as considerable spatial variation between our three core sites (see examples in Appendix 4 in Supporting information).

During counts we recorded 32,153 individuals belonging to 32 different Palearctic migrant bird species from 12 avian families (Supporting Table S2). The abundance of Palearctic migrants showed a strong peak in the southern African summer (Fig. 4), although the exact timing and magnitude of the peak varied between sites and years. Comparison of the abundance of Palearctic migrants and the prevalence of viruses from the same site and time, treating each site as an independent sample at each time step, found no dependency of viral prevalence on numbers of migrants (Spearman's $r = 0.039$, $P < 0.8$, $n = 42$). Viral prevalence was also independent of the numbers of anatid ducks (Spearman's $r = -0.1$, $P < 0.5$, $n = 42$). At time lags of 2 and 4 months, and excluding the Lake Ngami data, the relationship remained insignificant (2 months, Spearman's $r = 0.2$, $P < 0.22$, $n = 35$; and at 4 months, $r = 0.1$, $P < 0.57$, $n = 33$).

Discussion

The overall prevalence of LPAI influenza viruses that we found in anatid ducks across southern Africa is 2.4%. The range in PCR prevalence in anatids reported from Northern Europe is between 2.1% and 3.8% (Munster et al., 2006; Munster & Fouchier, 2009); and an extended survey in EU member states documented an overall LPAI prevalence in Europe of 1.87% (Breed et al., 2007). Some studies have found higher prevalences, ranging from 4% in Switzerland (Baumer et al., 2010) through 6.1% for European dabbling ducks (Munster et al., 2007) to as high as 12–15% (Wallensten et al., 2007; Terregino et al., 2007; Olsen et al., 2006). Estimates depend on the time of year when sampling occurred and the species that were tested (Olsen et al., 2006); our results are within the range of northern hemisphere estimates rather than notably lower.

One of our most interesting results is the lack of a predictable annual spike in prevalence. In Canada, for example, AIV prevalence in anatids may be as high as 60% on breeding grounds in early fall (Olsen et al., 2006). Our highest prevalence across all birds for any one sampling event was 21.43%, in summer in Zimbabwe; but in the same month in the following year, albeit with a relatively small sample size, prevalence was zero (Fig. 3). We attribute this unpredictability to the relatively stochastic nature of southern African seasonality and the flexible movement strategies of nomadic southern African ducks (Hockey, 2000; Hockey et al., 2004).

The prevalence of influenza A viruses in southern Africa appears to be twice as high in dendrocygnid (whistling) ducks (5.2%) as in anatid ducks (2.4%), although this result may be partly an artefact of whistling ducks having been sampled in largest numbers at the site with the highest overall virus prevalence. Most of our dendrocygnid samples were from White-faced Whistling Duck *Dendrocygna viduata*, but Fulvous Duck *Dendrocygna bicolor* are common (if almost uncatchable) at Lake Chuali and Lake Ngami. Whistling ducks are less abundant in South Africa but we observed both Fulvous and White-backed Duck *Thalassornis leuconotus* as far south as Strandfontein, and

Table 2. Information on Viral Strains and Types

Common name	Latin name	Family	Total +ves	H1 +ve	H3 +ve	H5 +ve	H6 +ve	H7 +ve	Typed
African Hoopoe	*Upupa africana*	Upupidae	1						
African Jacana	*Actophilornis africanus*	Jacanidae	15			2			
African Pipit	*Anthus cinnamomeus*	Motacillidae	1						
African Red-eyed Bulbul	*Pycnonotus nigricans*	Pycnonotidae	1			1			
African Snipe	*Gallinago nigripennis*	Scolopacidae	1						
African Wattled Lapwing	*Vanellus senegallus*	Charadriidae	3					1	
Barn Swallow	*Hirundo rustica*	Hirundinidae	1						
BlackSmith Lapwing	*Vanellus armatus*	Charadriidae	8			1		1	
Cape Teal	*Anas capensis*	Anatidae	1						
Chestnut-backed Sparrowlark	*Eremopterix leucotis*	Alaudidae	3			1			
Common Ringed Plover	*Charadrius hiaticula*	Charadriidae	1						
Common Sandpiper	*Actitis hypoleucos*	Scolopacidae	2						
Egyptian Goose	*Alopochen aegyptiaca*	Anatidae	7	1					H1N8
Fulvous Duck	*Dendrocygna bicolor*	Dendrocygnidae	2				1		
Glossy Ibis	*Plegadis falcinellus*	Threskiornithidae	1						
Helmeted Guineafowl	*Numida meleagris*	Numididae	1						
Hottentot Teal	*Anas hottentota*	Anatidae	3			1			
Little Rush-warbler	*Bradypterus baboecala*	Sylviidae	1						
Little Stint	*Calidris minuta*	Scolopacidae	3					1	
Malachite Kingfisher	*Alcedo cristata*	Alcedinidae	1			1			
Pied Kingfisher	*Ceryle rudis*	Cerylidae	1						
Red-billed Quelea	*Quelea quelea*	Ploceidae	1						
Red-billed Teal	*Anas erythrorhyncha*	Anatidae	35		1			2	H3N8
Red-knobbed Coot	*Fulica cristata*	Rallidae	7						
South African Shelduck	*Tadorna cana*	Anatidae	2			1	1		
Southern Grey-headed Sparrow	*Passer diffusus*	Passeridae	1						
Southern Masked-weaver	*Ploceus velatus*	Ploceidae	1						
Spur-winged Goose	*Plectropterus gambensis*	Anatidae	2						
Village Weaver	*Ploceus cucullatus*	Ploceidae	2						
White-faced Duck	*Dendrocygna viduata*	Dendrocygnidae	10			1		5	
Willow Warbler	*Phylloscopus trochilus*	Sylviidae	1						
Wood Sandpiper	*Tringa glareola*	Scolopacidae	1			1			
Yellow Bishop	*Euplectes capensis*	Ploceidae	1						
Yellow-billed Duck	*Anas undulata*	Anatidae	2						
Yellow-throated Longclaw	*Macronyx croceus*	Motacillidae	1						
Totals			125	1	1	10	2	10	(2)

This table describes birds that tested positive, rather than positive samples; the 6 birds that tested positive for the same type on two different swabs provide 6 entries rather than 12. Note that blank cells are zeros rather than unknown values.

Barberspan periodically hosts flocks of >20 White-faced Whistling Duck. Analyses of the movements of White-faced Whistling Duck in southern Africa suggest displacement on the scale of around 100 km/year, although ringing records suggest displacements of up to 1125 km and seasonally nomadic movements to ephemeral wetlands (Petrie and Rogers, 1997; Hockey et al., 2004; Underhill et al., 1999). White-faced Whistling Duck and Fulvous Duck have an extensive pan-African range and individuals from populations north of the equator may mix with Palearctic species, such as Garganey *Anas querquedula*, that migrate annually to western Europe. Gaidet et al. (2007) reported an AI prevalence of 3% in West African dendrocygnids and found HPAI H5 genomes in White-faced Whistling Duck in West

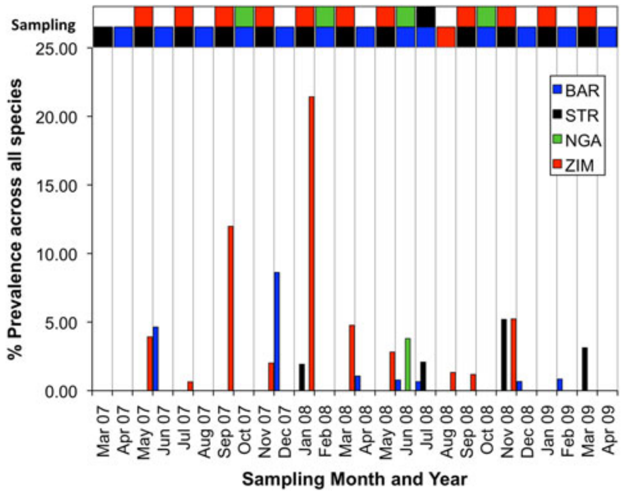

Figure 3. Prevalence of avian influenza by site and month across all captured birds. Sites are *BAR* Barberspan, *STR* Strandfontein, *NGA* Ngami and *ZIM* Zimbabwe (Manyame and Chivero). Note that (1) another 294 birds were sampled in Mozambique over the same period, with no AIV positives found; and (2) BAR, STR and ZIM were sampled every 2 months and NGA every 4 months, so birds were not sampled in some months. The *shaded squares at the top* of the chart indicate when a given site was sampled, using the same colour codes as the *bars*.

Africa. Given their high abundance and mobility (Cumming et al., 2008), whistling ducks may play an important regional role in the dynamics of AIV.

Sequencing and phylogenetic analysis of the H1N8 virus that was typed from Barberspan, together with other AIVs found in South Africa (Abolnik et al., 2010), yielded no evidence of internal genes associated with Asian HPAI H5N1 strains. The H1N8 virus from our study was the first isolate of an H1 AIV in southern Africa; its hemagglutanin gene grouped closely (96.4–97.4%) with homologous genes of Italian H1 N1 samples, suggesting a possible link to Europe (Abolnik et al., 2010).

Although no HPAI viruses were positively identified, potentially virulent H5 and H7 strains are in circulation in southern Africa in resident wild bird populations. Both viral abundance and the presence of potentially virulent strains appeared to be higher in the Manyame catchment, our northernmost site. There is some hint of a latitudinal gradient in prevalence, with Manyame > Barberspan > Strandfontein; but data from Mozambique and Botswana do not fit this pattern, although the sample sizes ($n = 271$ and 467 birds, respectively) are too small to draw strong inferences.

Most studies of avian influenza have focused on Anseriformes and Charadriiformes (ducks and waders), but other waterbirds may play a role in maintaining AIV in southern Africa. Rallids and jacanids (e.g. Red-knobbed Coot *Fulica cristata* and African Jacana *Actophilornis africanus*) occur year-round in high abundances in many wetlands and were frequently observed foraging near to dabbling and diving ducks. Cormorants and darters (Phalacrocoridae) are common in our study sites, mobile and frequently seen roosting with ducks. Risks of transmission to humans are increased by their capture in fishing

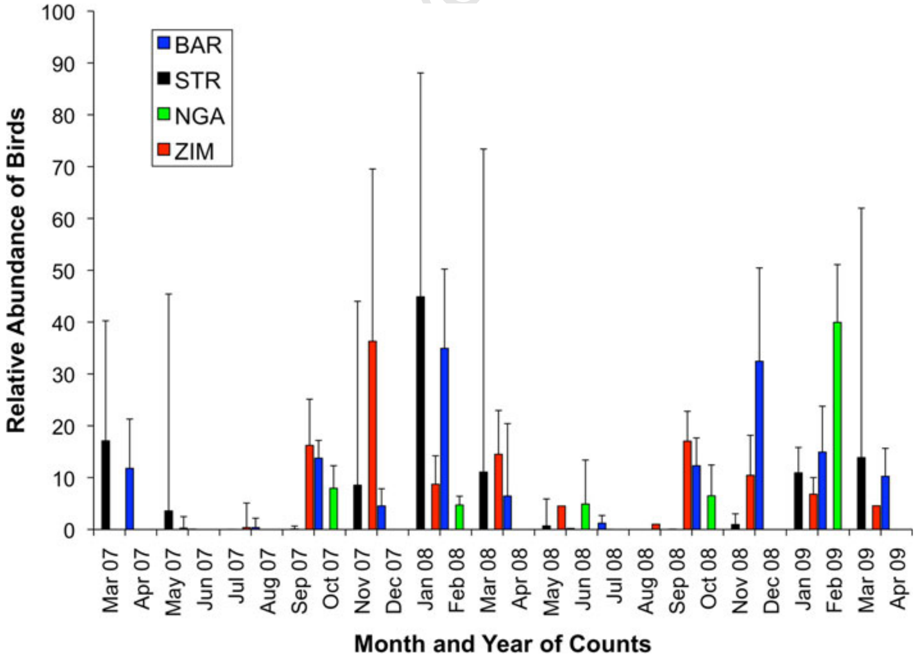

Figure 4. Relative abundance of Palearctic migrants per half-hour point count by site. *Bars* represent a mean number of migrants per point count; *error bars* represent an average standard deviation across point counts for all species for a given mission. A breakdown by species is given in Appendix 1 (Supporting information). Birds arrive earlier at more northern sites (ZIM and NGA); seasonal peaks coincide with the austral summer and the boreal winter.

nets (and indeed, we were able to sample a White-breasted Cormorant that survived an encounter with a fishing net on Lake Manyame). A variety of other species such as Sacred Ibises (*Threskiornis aethiopicus*; Threskiornithidae) also share foraging habitats with grazing and dabbling ducks (Hockey et al., 2004); Sacred Ibises in particular may feed on carcasses, making them potentially vulnerable during AIV epizootics in locations (e.g. unmonitored lakes) where carcass removal is not rapid.

For the Passeriformes, a prevalence of 4.5% (14 positives out of 308 birds) suggests a potential role in influenza epidemiology. Most of the AIV positive species that we found in this order are residents (Yellow-throated Longclaw, Chestnut-backed Sparrowlark, Red-billed Quelea and Village Weaver; Appendix 1 in Supporting information) but Barn Swallows and Willow Warblers are Palearctic migrants. Our data and those from other studies (e.g. Caron et al., 2010b) suggest that some passeriform families (e.g. Alaudidae and Ploceidae) may contribute to the persistence and spread of AIV in southern African ecosystems. Our results are unusual from a European perspective, suggesting higher prevalence than expected in southern African passerine populations, but agree with recent findings from the United States (e.g. Fuller et al., 2010) that imply a larger role for passerines in avian influenza dynamics than has been previously proposed.

In practical terms, our results preclude the assumptions of an annual cycle of virus circulation and strong seasonal variation in wild bird-related risks that hold in many northern hemisphere regions. From the perspective of both humans and poultry, AIV transmission by wild birds appears to be possible at any time of the year. The opportunistic behavioural responses of waterbird populations to environmental drivers, and the lag between rainfall and bird and pathogen responses, may nonetheless make it possible to obtain short-term predictions of AIV risks using information on rainfall.

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Understanding the ecological drivers of avian influenza virus infection in wildfowl: a continental-scale study across Africa

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Understanding the ecological drivers of avian influenza virus infection in wildfowl: a continental-scale study across Africa

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Despite considerable effort for surveillance of wild birds for avian influenza viruses (AIVs), empirical investigations of ecological drivers of AIV prevalence in wild birds are still scarce. Here we used a continental-scale dataset, collected in tropical wetlands of 15 African countries, to test the relative roles of a range of ecological factors on patterns of AIV prevalence in wildfowl. Seasonal and geographical variations in prevalence were positively related to the local density of the wildfowl community and to the wintering period of Eurasian migratory birds in Africa. The predominant influence of wildfowl density with no influence of climatic conditions suggests, in contrast to temperate regions, a predominant role for inter-individual transmission rather than transmission via long-lived virus persisting in the environment. Higher prevalences were found in *Anas* species than in non-*Anas* species even when we account for differences in their foraging behaviour (primarily dabbling or not) or their geographical origin (Eurasian or Afro-tropical), suggesting the existence of intrinsic differences between wildfowl taxonomic groups in receptivity to infection. Birds were found infected as often in oropharyngeal as in cloacal samples, but rarely for both types of sample concurrently, indicating that both respiratory and digestive tracts may be important for AIV replication.

Keywords: influenza A virus; pathogen transmission; disease ecology; wild birds; tropical; migration

1. INTRODUCTION

Understanding the influence of host ecology on the dynamics of pathogen transmission is currently recognized as fundamental to preventing and controlling wildlife infectious diseases [1,2]. Avian influenza viruses (AIVs) in wild birds have received increasing attention in recent years in response to the emergence and spread of the H5N1 highly pathogenic avian influenza (HPAI) virus across Eurasia and Africa [3,4]. Empirical investigation of the interface between the ecology and epidemiology of AIV in wild birds are, however, still in a relatively early phase of scientific exploration, and studies exploring the ecological interactions between AIV and their natural hosts are scarce [5–8]. In table 1, we present the potential ecological drivers of AIV

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Table 1. Potential ecological drivers of AIV prevalence in wild birds derived from experimental and empirical findings of the ecological interactions between AIV and wildfowl.

mechanisms	epidemiological predictions[1] and experimental[2] or empirical[3] findings	ecological factors of potential influence on AIV transmission
environmental transmission	[1]bird could be infected from long-lived virus persisting in the environment by drinking or feeding; infection rate depends on the virus concentration and persistence in the environment, and bird consumption rate [9] [2]AIV can remain infectious for several months in water under experimental conditions. Warmer temperatures, radiation and desiccation reduce the duration of AIV infectivity [10,11] [3]mathematical models of environmental transmission capture some patterns of AIV infection dynamics in wildfowl [9,12]	local climate may influence the environmental persistence of AIV
	[2]ducks can be successfully infected by contact with contaminated water [13] [3]higher prevalence is commonly reported in *Anas* species of dabbling ducks compared with diving or grazing wildfowl [3,5] [3]morphological trait associated with filtration of food particles (density of lamellae) has been positively associated with variations in AIV prevalence and diversity of subtypes shed in dabbling ducks [14]	species foraging behaviour may influence exposure to environmental infection
inter-individual transmission	[1]both transmission via airborne droplets or short-lived viruses shed in the environment are considered as essentially direct because they occur on the same time scale and rely on the proximity of hosts [9,12] [3]seasonal peak in prevalence in a wildfowl species that forage mainly on land [8], and higher AIV detection rate in respiratory than intestinal tract, support the existence of a direct airborne transmission via the respiratory route [4,8,15] [1]inter-individual transmission is expected to be density-dependent since the contact rate scales with host density [16] [3]northern autumn peak in AIV prevalence in ducks coincides with a seasonal social aggregation during pre-migration and migration that likely promotes contact rate and viral transmission [5,10,17,18]	host density and seasonal patterns of social aggregation may influence contact rate and transmission
host receptivity	[1]difference in prevalence between species may result from a difference in intrinsic receptivity to AIV infection [19] [3]the spectrum of AIV receptors on host cell surface vary substantially among different bird species [20]	taxonomic group may influence receptivity to infection

host susceptibility	[2]natural and experimental AIV infection stimulates the production of long-lasting AIV specific antibodies in ducks; subsequent exposure to AIV produce a boost in AIV antibody titers [21,22] [2]prior exposure to homo- or heterosubtypic AIV reduces the duration and concentration of viral shedding in consecutive infections, demonstrating the existence of a partial cross-protective immunity against re-infection [21,23] [3]natural consecutive infections with different AIV subtypes have been reported in ducks providing evidence that a prior exposure does not fully protect against a subsequent AI virus infection with a heterosubtypic AIV [6,24] [3]higher prevalence in hatch-year birds compared with after-hatch-year birds is consistently reported [5,8] [3]the duration of virus shedding decreases during the northern autumn in wild ducks [6]	geographical range associated with migratory behaviour and age may influence previous AIV exposure hence susceptibility to re-infection
population immunity	[1]the transmission rate depend on the proportion of susceptible individuals in the host population and the rate at which they experience their first infection [1,2,16] [3]prevalence strongly decline during northern autumn and winter as the proportion of immunologically naive hatch-year birds progressively decreases through infection or as result of a greater mortality rate compared with adults [5,17,18,25]	demographic rates and seasonal peaks in prevalence may influence the turnover of susceptible hosts
host dispersal	[1]infected hosts shedding virus may disperse AIV as they move [2,10] [2,3]experimentally [21,26] and naturally infected wildfowl [6] generally excrete AIV for one to three weeks without clinical signs or lesions [3]migratory wildfowl are able to perform long-distance movements within the time frame of AIV infection [27] [3]phylogenetic analysis confirms the occurrence of inter-continental exchange of AIV [28] [3]phylo-geographical clusters of AIV in wildfowl across North America suggest a dominance of introduction over persistence in the interannual perpetuation of AIV [29]	timing and range of migration may influence period and origin of virus introduction

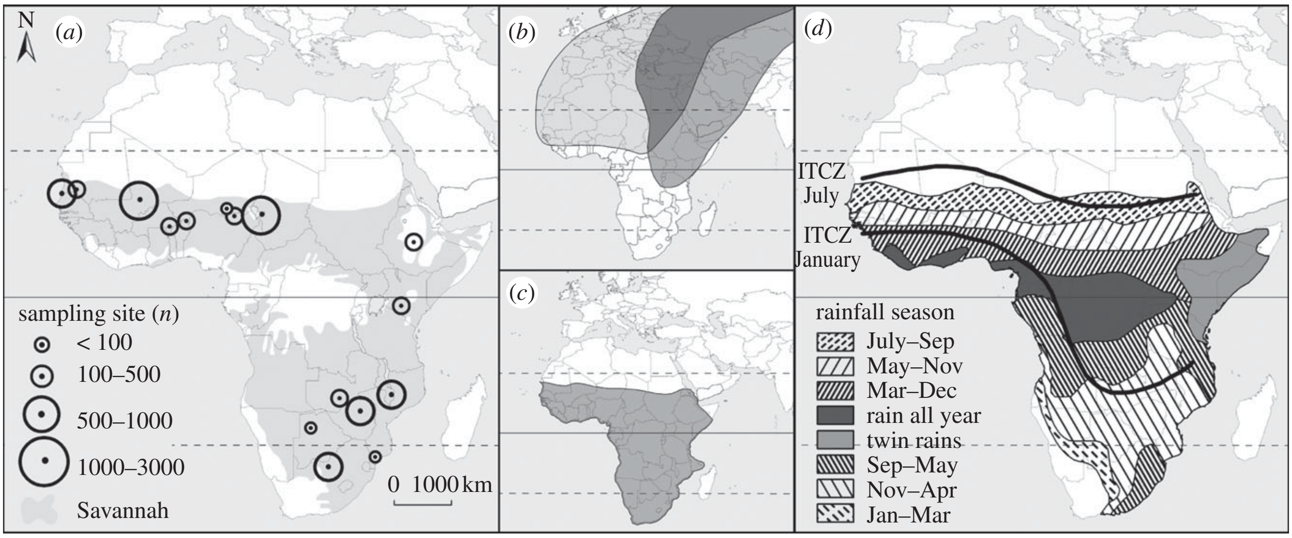

Figure 1. (*a*) Location of study sites (number of birds sampled); (*b*) main migratory flyways, and distribution range of Eurasian wildfowl and (*c*) Afro-tropical wildfowl in sub-Saharan Africa, adapted from [30]; (*d*) timing of the wet season and seasonal position of the Inter-Tropical Convergence Zone (ITCZ) adapted from [31].

prevalence in wild birds based on a review of our current knowledge of the mechanisms, whereby host ecology and the environment may influence AIV transmission in wild birds [1–29]. Despite the investment of considerable effort in AIV detection in wild birds, there is a lack of data-heavy empirical tests, in particular across vast geographic areas, of the influences of these ecological drivers on AIV transmission [4].

To test a range of current assumptions and hypotheses about the ecological drivers of AIV prevalence, we investigated the relative roles of a range of ecological factors, including species traits, migration patterns, climate and seasonal fluctuations in the abundance and composition of the host community, on patterns of AIV prevalence in their main natural reservoir and wildfowl (*Anseriformes*). We explored relationships across a variety of environmental conditions and host communities using a continental-scale dataset from 15 African countries (figure 1*a*; electronic supplementary material, table S1).

It is important to note that most of our understanding of the ecology of AIV is derived from studies that have been conducted in boreal or temperate regions of the Northern Hemisphere (table 1). There is a knowledge gap in tropical regions, particularly in sub-Saharan Africa [3]. Yet, earlier studies have suggested that tropical regions may act as epicentres contributing to year-round AIV perpetuation in wild birds [10]. More recently, AIVs have been found circulating in wild birds across Africa [32–35] indicating that local environmental conditions are favourable for AIV transmission. However, the patterns of AIV prevalence observed in temperate or boreal regions cannot be directly transposed to the tropics where differences in host ecology, climate and seasonality may produce different dynamics of infection.

Eurasian (i.e. Palaearctic-breeding) migratory wildfowl winter in large numbers between September and March in Afro-tropical regions north of the equator [30,36] (figure 1*b*). All African regions also host an influx of Eurasian migrants from other waterbird groups including waders, gulls, terns, rails, herons and storks. In their wintering sites, Eurasian wildfowl mix with Afro-tropical wildfowl that reside year-round within sub-Saharan Africa (figure 1*c*). Afro-tropical wildfowl generally breed during or following the wet season but their breeding season is often much more extended than in Eurasian wildfowl, with laying periods stretching over 6–12 months for a given area [37]. In Afro-tropical regions, seasons are determined by rainfall rather than temperatures, which are higher and exhibit lower seasonal variation than in temperate regions. The duration of the wet season varies according to latitude with an asynchrony in the timing of rainfall between regions north and south of the equator (figure 1*d*). Most Afro-tropical wetlands, a key habitat and resource for wildfowl, experience extreme seasonal variations in their surface area: relatively short but intense rains and extensive river flooding can inundate vast floodplains [36], while high evaporation rates and human extraction of water drastically reduce the extent of wetlands during the dry season. The intensity of these seasonal drivers could be of great local importance: in the Inner Niger Delta in Mali, for instance, the surface area of seasonally flooded wetlands may be up to 20 times higher than the surface area of permanent wetlands [36].

Given the numerous differences between tropical and temperate ecosystems, we hypothesized that the mechanisms whereby host ecology and the environment influence AIV transmission in Afro-tropical regions should operate through ecological drivers derived from the context of Afro-tropical ecosystems. The presumably low environmental persistence of AIV under tropical climates might result in a predominant influence of ecological factors such as host density that are associated with inter-individual transmission (i.e. transmission through airborne droplets via the respiratory route or through short-lived viruses shed in the environment via the faecal–oral route), over climatic factors that are associated with environmental transmission (i.e. via long-term persisting virus in an environmental reservoir). In Palaearctic and Nearctic regions, the northern autumn peak in AIV prevalence consistently observed in ducks [5,10,17,18] has been related to the congregation of ducks at pre-migration and migration-staging sites, at a time when populations consist of a large proportion of first-year immunologically naive birds [5,10,25]. The

Table 2. Definition of the explanatory variables, presented in six categories of variables found to be associated and tested alternatively by permutation in models.

explanatory variables		definition (units)
species traits	geographical origin	Eurasian versus Afro-tropical spp.
	taxonomic group	*Anas* versus non-*Anas* spp.
	foraging behaviour	prim. dabbling versus non-prim. dabbling (i.e. mostly grazing or diving)
	origin × taxonomy	Eurasian *Anas*, Afro-tropical *Anas*, Afro-tropical non-*Anas* spp.
	origin × foraging	Eurasian prim. dabbling, Afro-tropical prim. dabbling, Afro-tropical non-prim. dabbling spp.
	taxonomy × foraging	*Anas* prim. dabbling, non-*Anas* prim. dabbling, non-*Anas*-non prim. dabbling spp.
wildfowl density	species	no. birds of the species sampled or of the entire wildfowl community per area of wetland (bird km^{-2})
	community	
wildfowl community composition	proportion of Eurasian spp.	percentage of birds from Eurasian, *Anas* or prim. dabbling spp. in the wildfowl community
	proportion of *Anas* spp.	
	proportion of dabbling spp.	
climatic conditions	maximum annual temperature	annual or monthly mean of maximum daily temperature for the month of sampling (°C)
	maximum month temperature	
	annual PET	annual or monthly mean of daily potential of evapo-transpiration (PET) (mm), computed as a function of radiations, humidity, air temperature and wind speed
	monthly PET	
	aridity index	ratio of annual rainfall to PET (i.e. deficit of available water)
season	timing relative to the arrival of Eurasian migrants	no. of days between the median sampling date and 1 September
	timing relative to the end of the dry season	no. of days between the median sampling date and the end of the previous dry season
sampling method		single cloacal, single oropharyngeal or both swabs

resulting increase in the level of population immunity has been proposed to be a determinant of seasonal variations in AIV prevalence [5,19,25]. Analogously in the tropics, seasonality in AIV prevalence may be related to the congregation of wildfowl at permanent wetlands at the end of the dry season in response to the drying of wetlands. Another potential driver of AIV seasonality in Africa is the arrival of Eurasian migratory waterbirds, which represent a potential source of virus introduction (table 1).

Species variation in AIV prevalence is commonly reported between cohabiting wildfowl species [5,14]. A difference between Eurasian and Afro-tropical wildfowl in previous AIV exposure at breeding or migration-staging grounds may imply a difference in susceptibility to re-infection between these species when they co-habit in Africa. The higher AIV prevalence consistently reported in dabbling ducks (i.e. surface and shallow water foragers) of the *Anas* genus compared with other wildfowl species [3,5] suggests a potential heterogeneity in host competence among wildfowl species from different ecological guilds or taxonomic groups. These differences are commonly associated with foraging behaviour, with surface/shallow water feeders being more exposed to water-borne infection than divers or grazers ([3,5,19]; table 1). However, it may also result from a difference in receptivity to AIV infection among host species [19], determined by species-specific differences in the type of AIV receptors present on epithelial tissues [20]. Consequently, the proportion of the most competent host species present in a wildfowl community may have a substantial influence on the capacity of the community to perpetuate AIV.

While dabbling ducks in the Palaearctic and Nearctic are largely represented by *Anas* species, the dabbling ducks community in sub-Saharan Africa comprises a greater proportion of non-*Anas* species, particularly the abundant and widely distributed whistling ducks (*Dendrocygna* spp.). Afro-tropical regions north of the equator are characterized by a strong seasonal influx of Eurasian wildfowl, which are largely absent in the regions south of the equator. In contrast with East and Southern Africa, Afro-tropical *Anas* species are rare in West Africa, where *Anas* species are represented almost exclusively by Eurasian migratory ducks [30,36,37]; consequently, *Anas* species are scarce during half of the year in West Africa. These specificities and regional differences in the composition of the wildfowl community across sub-Saharan Africa provide the opportunity to tease apart in our continental study the respective influence of migratory patterns, foraging behaviour and taxonomy on species prevalence.

In this study, we used generalized linear mixed models and a model comparison approach to assess the ability of various ecological factors (table 2) to explain species, seasonal and geographical variations in AIV prevalence measured in wildfowl across Afro-tropical regions. We tested factors related to: (i) the probability of a wildfowl species being infected, including its migratory and foraging behaviours and taxonomic group; and (ii) the capacity of the local host community and environment to perpetuate the virus, including the host density (at species and wildfowl community level), the proportion of the potentially most competent species (Eurasian, dabbling or *Anas* species) in the wildfowl community, the climate (temperature and aridity indices) and the timing of sampling relative to the arrival of Eurasian migrants or to the dry season.

2. MATERIAL AND METHODS

(a) *Sampling and avian influenza virus detection procedures*

Free-living wildfowl were sampled between 2006 and 2009 at 16 sites, all permanent wetlands selected from among the most important waterbird areas within the study region (electronic supplementary material, table S1) [30]. Sampling was conducted on a different number of occasions between sites and years (electronic supplementary material, table S2), with at least two months between sampling occasions in any given site. All samples were collected using cotton swabs and immediately stored in cryovials containing a viral transport medium. Birds were tested for AIV infection using three distinct sampling methods that we distinguished in our subsequent analyses: a single cloacal swab, a single oropharyngeal swab or both cloacal and oropharyngeal swabs tested individually. Samples were analysed in different laboratories using a similar standard diagnostic procedure based on RNA extraction and real-time RT-PCR virus detection (see electronic supplementary material, SI methods, for a complete description of sampling and diagnostic procedures). We computed the observed prevalence for each species for each sampling occasion as the percentage of individuals found positive for AIV compared with the total number of birds tested.

(b) *Explanatory variables*

Ground-based and satellite-based data were used to estimate the values of six categories of explanatory variables listed in table 2. Continuous variables (wildfowl density, community composition, climatic conditions and timing of sampling) were estimated for each sampling occasion. Details about data source are provided in electronic supplementary material (electronic supplementary material, SI methods).

(c) *Analysis*

Measures of bird density were log-transformed and standardized, together with measures of proportion of wildfowl species and climatic variables, to have a mean of 0 and an s.d. of 1. We investigated the potential association between variables using the Pearson correlation coefficient for continuous variables and the phi coefficient for categorical variables. Following Graham [38], pairs of variables with a correlation coefficient greater than or equal to 0.28 were considered associated and were tested separately in models. Multi-collinearity was high among variables representing alternative measurements of wildfowl density, wildfowl community composition, climate or the timing of sampling but not between these categories of explanatory variables. The three variables related to species traits (origin, taxonomic group and foraging behaviour) also showed a strong association as some combinations of the categories of these variables were not represented in our sample. Non-*Anas* or non-primarily dabbling wildfowl species are rare among the Eurasian wildfowl wintering in sub-Saharan Africa [30,36,37] and were consistently absent from our samples. Similarly, there were no non-primarily dabbling *Anas* species in our samples. In order to nonetheless build models where the effects of two of these interdependent categorical variables reflecting species traits were simultaneously accounted for, we generated three composite variables that combined pairs of variables (table 2).

We investigated the relationships between AIV prevalence and explanatory variables using a generalized linear mixed model and assuming a binomial distribution. The 55 distinct sampling occasions were distributed over 16 sites and 4 years. There were thus several sampling occasions within a given year and usually within a given site. In order to tackle this potential pseudo-replication issue, a year and a site random effect were included in the models. The potential aggregations of infected birds within sampling occasions was also accounted for by incorporating the sampling occasion as a random effect nested within year and site. Finally, we included a random laboratory effect to account for a potential difference in diagnostic sensitivity among laboratories. Models were run with the 'glmer' function in the 'lme4' package in the R environment, using Laplace approximation of the maximum-likelihood and a logit link function. We used an information-theoretic procedure and the Akaike information criterion corrected for small sample sizes ($\mathrm{AIC_c}$) to compare models [39].

Our analysis consisted of three steps. We first selected among alternative explanatory variables that were found to be associated by testing them successively by permutation in models. We generated a first set of models that consisted of all the combinations of explanatory variables where a single variable was included for each of the six independent categories of variables ($6 \times 2 \times 3 \times 5 \times 2 \times 1 = 360$ models). The relationships between prevalence and the two variables related to the timing of sampling (t) were considered as cyclic and were modelled using a cosine function of the form $\mathrm{logit(prevalence)} = a + \cos(2\pi t/365)$. All the models considered at this step of the analysis included the fixed additive effects of six explanatory variables and the random effects of year, site, sampling occasions and laboratory. For each model i, we computed the Akaike weight (ωi) which can be interpreted as the likelihood that model i is the best model within the set in terms of trade-off between fit to the data and parsimony. For each independent category of explanatory variables, we selected for the next step of the analysis the variable that yielded the highest sum of Akaike weights ($\Sigma\omega i$), computed for all models in the set in which that variable occurred [39].

In the objective of obtaining a minimal adequate model, i.e. a model including only important effects, and of assessing the statistical support associated with each random effect, we explored in a second step the random part of the model. We compared the variance components associated with each of the four random factors (year, site, sampling occasions and laboratory) in models that included as fixed effects the six explanatory variables previously selected. We iteratively removed from the model the random factors with the lower variance components by comparing $\mathrm{AIC_c}$s between models that included or excluded this random factor.

Finally, we evaluated the relative importance of each of the six independent explanatory variables retained after the initial selection by comparing $\Sigma\omega_i$ among models which included or excluded the effect of this variable. We created a second set of models which contained all possible combinations that could be generated by including or excluding each of the six independent explanatory variables as fixed effects ($2^6 = 64$ models) and computed the $\Sigma\omega_i$ for each variable. We computed the coefficients of model parameters through model averaging across all models having $\Delta\mathrm{AIC_c} < 2$ (compared with the model with the lowest $\mathrm{AIC_c}$), weighting coefficients of model parameters by the model's Akaike weight and summing the weighted coefficients [39].

Table 3. Summary of the three best-supported models ($\Delta AIC_c < 2$) fitted to estimate variations in AIV prevalence in wildfowl in Afro-tropical regions, with coefficient estimates (±s.e.) and relative importance of selected explanatory variables ($\Sigma\omega_i$). All models were fitted as generalized mixed effects models, with sampling site and occasion fitted as random intercept terms and other explanatory variables as independent fixed effect. *k*, number of estimable parameters; AIC_c, Akaike's information criterion for small samples; ω, Akaike weights; $\Sigma\omega i$, relative importance of each explanatory variable estimated by summing the Akaike weights of all models in the set where that variable occurred.

model	*k*	AIC_c	ω	intercept	taxon. group non-*Anas* spp.	wildfowl community density	timing/arrival Eurasian migrants[a]	sampling method		maximum month temperature	proportion of Eurasian wildfowl spp.	random effects[b]	
								cloacal + oropharyngeal	single oroph.			site	occasion
1	8	265.6	0.31	−3.95 ± 0.35	−0.97 ± 0.17	0.53 ± 0.21	−0.68 ± 0.27	0.47 ± 0.23	−0.36 ± 0.47			0.67	0.43
2	9	267.0	0.15	−3.90 ± 0.35	−0.96 ± 0.17	0.52 ± 0.21	−0.71 ± 0.27	0.45 ± 0.23	−0.38 ± 0.47	−0.18 ± 0.22		0.70	0.41
3	9	267.5	0.12	−3.95 ± 0.35	−0.96 ± 0.17	0.51 ± 0.21	−0.67 ± 0.29	0.48 ± 0.23	−0.36 ± 0.47		0.12 ± 0.26	0.69	0.43
average				−3.94 ± 0.35	−0.97 ± 0.17	0.52 ± 0.21	−0.67 ± 0.27	0.47 ± 0.23	−0.36 ± 0.47	−0.18 ± 0.22	0.12 ± 0.26		
$\Sigma\omega i$					1.00	0.88	0.87	0.83		0.34	0.32		

[a] Relationship modelled with a cosine function; [b] random effect variance estimation.

3. RESULTS

(a) *Avian influenza virus detection*

We sampled and tested a total of 8413 free-living wildfowl of 18 species (electronic supplementary material, table S3). AIV were detected in 3.3% ($n = 278$) of birds tested, in almost all countries (except Burkina Faso, Kenya and Mozambique), and in all species for which more than 31 birds were sampled (electronic supplementary material, table S3). The proportion of AIV-positive birds found was highly variable between species, sites and sampling occasions, reaching up to 14.7 per cent ($n = 225$) in garganey (*Anas querquedula*) in Mauritania in February 2006.

(b) *Ecological factors related to avian influenza virus prevalence*

The initial selection among alternative explanatory variables within each independent categories of variables (table 2) indicated that the best predictors of AIV prevalence according to Akaike relative importance weights were (electronic supplementary material, table S4): (i) taxonomic group ($\Sigma\omega_i$ of the models with this variable $= 0.50$) compared with five other species traits tested separately or in pairs (all $\Sigma\omega_i \leq 0.26$); (ii) wildfowl density at the community level ($\Sigma\omega_i = 0.92$) compared with the density at the species level; and (iii) timing of sampling relative to the arrival of Eurasian wildfowl ($\Sigma\omega_i = 0.84$) rather than relative to the end of the dry season. The alternative variables related to wildfowl community composition or climate had Akaike weights of relatively similar importance: the proportion of Eurasian wildfowl ($\Sigma\omega_i = 0.40$) and the mean maximum temperature of the month of sampling ($\Sigma\omega_i = 0.24$) presenting the highest $\Sigma\omega_i$ were retained in the subsequent analyses.

The analysis of the dependence structure of our dataset indicated that the random effect of the site and the sampling occasion accounted for most of the variance of the random part, and the exclusion of the random effect of laboratory or year reduced the AIC_c of the mixed models ($\Delta AIC_c > 2$). The site and the sampling occasion were thus included as the only random effects in all subsequent models.

The AIC_c-based comparison procedure of the relative importance of each of the six independent explanatory variables retained after the initial selection indicated that four variables were important in explaining the variation in AIV prevalence (table 3). The high Akaike importance weights of the species taxonomic group ($\Sigma\omega_i = 1$), the density of the wildfowl community ($\Sigma\omega_i = 0.88$), the timing of sampling relative to the arrival of Eurasian migrants ($\Sigma\omega_i = 0.87$) and the sampling method ($\Sigma\omega_i = 0.83$) indicate that these variables occurred in all high ranking models. The three best-supported models ($\Delta AIC_c < 2$) all included these four variables (table 3), and fitted the data adequately (Pearson χ^2 goodness-of-fit test = 240.2–243.8, ddl = 265, $p = 0.82$–0.86, H0: 'the model fits the data' cannot be rejected). Inclusion of variables associated with the proportion of Eurasian wildfowl species ($\Sigma\omega_i = 0.32$) or the mean maximum temperature of the month of sampling ($\Sigma\omega_i = 0.34$) received much less support from the data.

The coefficients estimated from the top three models (table 3) indicate that prevalence was higher in *Anas* species than in non-*Anas* species. A similar difference

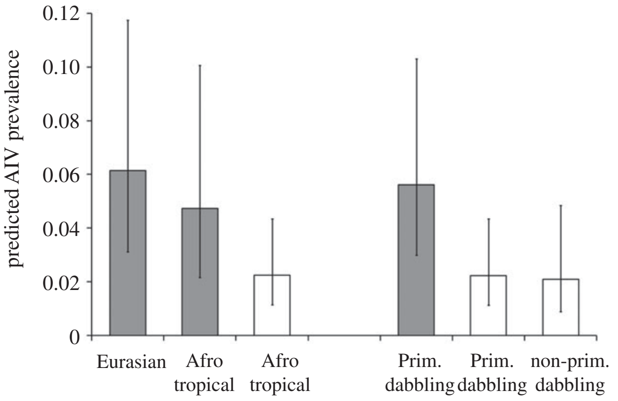

Figure 2. Mean AIV prevalence estimated for *Anas* (shaded bar) and non-*Anas* species (unshaded bar) of wildfowl belonging to distinct migratory groups and ecological guilds. Prevalences (95% CI, bars) were estimated for birds tested concurrently for cloacal and oropharyngeal samples, based on the highest rank model (table 3) after substituting the variable Taxonomic group by the composite variables Origin-Taxonomic group or Foraging behaviour-Taxonomic group. Other predictor variables were set to their mean value over the dataset.

was found between the two taxonomic groups when we accounted for the geographical origin or the main foraging behaviour of species (figure 2): *Anas* species showed a significantly higher prevalence than non-*Anas* species among Afro-tropical wildfowl (Z value $= 2.45$, $p = 0.014$) as well as among primarily dabbling wildfowl (Z value $= 5.17$, $p < 0.001$). These results are in agreement with the lower $\Sigma\omega_i$ found in the composite variables when compared with the variable taxonomic group tested separately (electronic supplementary material, table S4).

Prevalence was positively associated with the density of the wildfowl community (figure 3; rug plots illustrate the distribution of data points along the x-axis). Seasonal variation in AIV prevalence was related to the timing of arrival of Eurasian migrants: the prevalence progressively increased from the time when Eurasian migrants arrive in Afro-tropical regions (September) to peak at the end of their wintering period (February–March), and decreased after their departure (figure 3). Seasonal variation in AIV prevalence was poorly described by models including the effect of the timing relative to the end of the dry season. Substituting this variable with the timing relative to the arrival of Eurasian migrants substantially increased the AIC_c value of the best-supported model ($\Delta\text{AIC}_\text{c} = 4.8$).

Finally, prevalence was higher for birds tested concurrently for both cloacal and oropharyngeal samples than in birds tested for a single cloacal sample (Z value $= -2.20$, $p = 0.028$). However, prevalence was similar between birds tested for a single cloacal or a single oropharyngeal sample (Z value $= -0.66$, $p = 0.509$). A similar trend was found for both the groups of *Anas* and non-*Anas* species (electronic supplementary material, figure S1). In addition, among birds tested for both cloacal and oropharyngeal samples individually ($n = 3075$), few birds were found positive concurrently for both types of sample ($n = 6$), while birds were found positive as frequently from cloacal samples only ($n = 55$) as from oropharyngeal samples only ($n = 46$) (McNemar test, $p > 0.05$).

4. DISCUSSION

Our results indicate that variations in AIV prevalence in wildfowl at a continental scale were related to several host ecological factors operating at both species and community level, including the species taxonomic group, the local density of the wildfowl community and the season when Eurasian migratory birds winter in Africa. The timing relative to the dry season congregations, the composition of the local wildfowl community and the climatic variables were relatively poor predictors of AIV prevalence. It also appears that sampling the respiratory tract may be as important as sampling the digestive tract to detect AIV infection in wildfowl. We consider each of these points in more detail in the following paragraphs.

Prevalence was positively related to the density of wildfowl measured at the community rather than at the species level, suggesting aggregation of infection through interspecies mixing. The density of the wildfowl community varied widely between sites and seasons (up to 3 log units, electronic supplementary material, table S1), in relation to the seasonal variations in wetlands surface and the massive flux of Eurasian migratory wildfowl but also Afro-tropical wildfowl congregating at permanent wetlands during the dry season or, conversely, with the dispersal of birds to newly flooded wetlands after the onset of the wet season. The proportion of Eurasian wildfowl in the wildfowl community was poorly related to the variations in AIV prevalence suggesting that the influx of Eurasian wildfowl influences AIV transmission by increasing the local wildfowl density but that the geographical origin of birds may not matter much. We thus found no support to our initial prediction of a potential difference between Eurasian and Afro-tropical wildfowl in previous AIV exposure and susceptibility to re-infection, neither at the community level (proportion of Eurasian species) nor at the species level (species origin, figure 2).

Climatic conditions varied widely between season and between our study sites: these sites stretched over four aridity classes (from arid to humid), with local monthly and annual means of maximum daily temperatures varying between 20°C–39°C and 25°C–36°C, respectively (electronic supplementary material, table S1). Climatic variables associated with a reduced survival of the virus in the environment were, however, poorly related to AIV prevalence. Afro-tropical regions are characterized by mean monthly temperatures of greater than or equal to 20°C in all months (except in African highlands), in contrast with boreal and temperate regions that are characterized by mean monthly temperatures less than 20°C during at least eight months per year. Maximum daily temperatures in most Afro-tropical regions may be over a threshold throughout most of the year where high temperatures prevent the perpetuation of AIV in the environment by more than a few days [11]. The positive association, which we found between AIV prevalence and the local wildfowl density with no influence of climatic conditions, suggests a predominant role of direct inter-individual transmission via the respiratory route [8] or via short-lived viruses recently shed in the

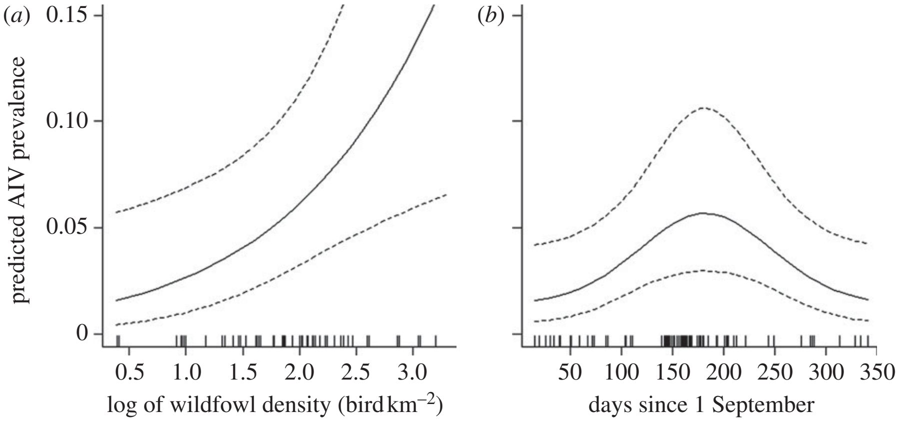

Figure 3. Predicted AIV prevalence (95% CI, dashed lines) for *Anas* species of wildfowl sampled across Afro-tropical regions in relation to the density of the wildfowl community and the timing relative to the arrival of Eurasian migrants (1 September used as a reference date). Prevalences were estimated for birds tested concurrently for cloacal and oropharyngeal samples, based on the highest rank model (table 3), with other predictor variables set to their mean value over the dataset. The distribution of data points is presented as rug plots along the *x*-axis (a vertical bar for each sampling occasion).

environment, rather than an indirect transmission via viruses persisting in the environmental reservoir. By contrast, in temperate regions theoretical models of AIV dynamics suggest a greater role for indirect environmental transmission than for density-dependent transmission [9,13].

Surprisingly, seasonal variations in prevalence were poorly related to the timing of congregation of wildfowl at the end of the dry season. In Palaearctic and Nearctic regions, concentration of wildfowl births into a short seasonal breeding period generates a pulse of immunologically naive birds into the host population [1,2]. The congregation of first-year susceptible birds during a relatively short autumn migration period, only a few months after the breeding season, probably increases the rate at which susceptible birds experience their first infection, producing a rapid increase in the level of population immunity. In Afro-tropical regions, extended breeding seasons produce a more gradual recruitment rate of juveniles into the host populations. The seasonal congregation of wildfowl in the dry season in the tropics is also more progressive than the northern migration flocking as it results from the progressive drying of wetlands while migration flocking results from a social gathering behaviour. These extended breeding seasons and progressive seasonal congregation may slow down the turnover rate of susceptible birds in the wildfowl community. The seasonality of AIV prevalence in our study (figure 3) was accordingly much less pronounced than in Europe (0–25%) [18] or North America (0–60%) [3]. This should reduce the controlling effect of population immunity on AIV transmission and promote a lower but continuous annual circulation as observed in a southern African wetland [34].

Our results indicated that AIV prevalence increases during the period when Eurasian migratory waterbirds (including non-wildfowl species) winter in sub-Saharan Africa and decrease after they migrate back to Eurasia. The arrival of Eurasian migrants constitutes a massive influx of hosts in the local waterbird community but also a potential source of AIV introduction. Eurasian wildfowl are largely absent in the regions south of the equator but large numbers of other Eurasian waterbird species, in particular shorebirds (Charadriiformes), winter in southern Africa. The role of shorebirds in the ecology of AIV is still unclear with highly contrasted results from Nearctic and Palaearctic regions [5,17]. A low prevalence has been reported globally in non-wildfowl species (less than 2%) [3,7] suggesting that they play a lesser role in the perpetuation of AIV, though locally shorebirds may have a significant role [7]. Phylogenetic analyses also indicate that inter-continental transfer of AIV genes, though occasional, do occur in shorebirds [40].

Difference in prevalence between species was better explained by the taxonomic group than by the foraging or the migratory behaviour of species. *Anas* species had higher prevalence than non-*Anas* species even when we account for difference in foraging behaviour or geographical origin of birds (figure 2). These results support the hypothesis [19] that there might be intrinsic differences between wild bird species, including between wildfowl taxonomic groups, in their receptivity to AIV infection. Dabbling ducks of the *Anas* genus are commonly reported to be more frequently infected than other wildfowl including grazing (*Anser*, *Branta* or *Cygnus* spp.) or diving wildfowl (*Aythya* spp.) [3,5,19]. Looking more closely at global AIV surveillance results reveal that non-*Anas* species of ducks that also forage primarily by dabbling in surface and shallow water (e.g. wood duck *Aix sponsa* and common shelduck *Tadorna tadorna*) have globally a lower prevalence than dabbling ducks of the *Anas* genus [3,5]. Similarly, differences in clinical disease and mortality to H5N1 HPAI virus infection has also been reported between *Anas* species (mallard *Anas platyrhynchos*, northern pintail *Anas acuta*, common teal *Anas crecca*) and non-*Anas* species of dabbling ducks (wood duck, muscovy duck *Cairina moschata*, ruddy shelduck *Tadorna ferruginea*, mandarin duck *Aix galericulata*) that had been concurrently experimentally inoculated [41–43]. A difference has been found between chickens and ducks in the distribution of sialic acid receptors of AIV on host epithelial tissues [20,44]; such a difference may also exist among different wildfowl species and limit interspecies transmission. Despite large differences in the proportion of *Anas* species in the wildfowl community between our study sites and seasons (1–96%, electronic supplementary material, table S1), variations in prevalence were poorly related to this variable. This suggests that the absolute rather than the relative number of birds from *Anas* species may influence AIV transmission.

In our study, the detection rate of AIV was similar in oropharyngeal and in cloacal samples and testing birds for both types of sample produced higher infection rates since birds were rarely found concurrently infected for both types. These results highlight the role of the respiratory tract for the replication of AIV. The traditional assertion that AIV replicate preferentially in the cells lining the intestinal tract of birds and are shed in their faeces [3,10] has been recently challenged by contrasted findings from several comparative studies reporting either a higher AIV detection rate from oropharyngeal samples [4,8,16] or from cloacal samples [26,45,46], or no difference in detection rates between these two sampling sources in wildfowl [4]. Moreover, experimental infection studies with H5N1 HPAI viruses have all evidenced a predominant oropharynx excretion in a diversity of wildfowl species (see the study of Gaidet *et al.* [27] for a review). The preferential site of replication may differ between species [4] and may be related to species' main foraging behaviour [8]. In our study, we found similar AIV detection rates in oropharyngeal and in cloacal samples in both *Anas* and non-*Anas* species (electronic supplementary material, figure S1). Though the exact role of a preferential oropharynx excretion on the dynamics of AIV transmission in wildfowl remains to be elucidated, the collection of oropharyngeal samples is essential for the field study of AIV in wild birds.

Our results provide a unique contribution to our understanding of the ecology of AIV in wild birds in tropical ecosystems but also offer a number of novel insights for understanding the general influence of seasonal fluctuations in animal density and migration on infectious disease dynamics. In addition, our approach illustrates the value of integrating ecology and epidemiology for understanding complex multi-host epidemiological systems. As our analysis shows, research at the interface between ecology and epidemiology could benefit hugely from cross-disciplinary inter-group data sharing and detailed empirical analyses of geographically diverse datasets.

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