Challenges within the annual cycle of long-distance migratory waders along the East Asian-Australasian Flyway

by

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Abstract

The East Asian-Australasian Flyway (EAAF) has the highest number of threatened waterbird species of all flyways in the world, the decline in many species being fore mostly attributed to habitat loss and deterioration. However, besides major perturbations of migratory stop-over sites along the east and SE Asian coast, it cannot be denied that conflicts with man are also on the increase at their Australian wintering grounds (main non-breeding ground) and that climate change is having a notably impact on these shorebirds’ Arctic breeding grounds.

Adopting a somewhat holistic approach, this thesis investigates the challenges that migratory waders (‘shorebirds’) along the EAAF face over the course of their annual cycle. Using data from shorebirds caught at different Australian wintering areas (i.e. King Island [Tasmania], southeast Australia [South Australia and Victoria] and Broome [north Western Australia]) and the utilisation of a range of techniques such as assessment of age-composition, stress biomarkers, parasite infection rates, tracking devices and modelling, we assessed the various challenges the birds are facing and made predictions on their potential capacity to cope with future changes.

In chapter 2, using juvenile percentages assessed on the shorebirds’ migratory staging and wintering sites, we show that the breeding success of high Arctic nesting waders is primarily influenced by summer temperatures in the Arctic. Moreover, we demonstrate that weakening lemming cycles, which had an indirect effect on breeding success of waders and geese in the past, have triggered a change to once cyclical breeding success in waders from the EAAF as well as from the East Atlantic Flyway. As there was no obvious decreasing trend in the breeding success of waders, the downward trend observed for many species is assumed to be a result of deteriorating conditions at stop over and/or wintering sites.

The rate of fuel deposition importantly determines a migrant’s speed and success of migration. In chapter 3 we show that fuelling rates are correlated with latitude, increasing from the tropics to the poles. In accordance with the “time minimization hypothesis”, this latitudinal trend has an effect on the migration strategy of birds flying from Australia to their Arctic breeding grounds. They typically fly in one go, across the equator, skipping low quality habitats in the tropics to arrive at high quality sites at higher latitudes, making small hops from thereon. This long non-stop journey requires appropriate preparation and it therefor highlights the disproportional reliance of long distance migratory waders on sites at high latitudes (i.e. southern Australia and temperate environments in the northern
hemisphere). Stochastic dynamic modelling predictions (chapter 4) suggested that birds that failed to fuel sufficiently at the high latitude wintering site in South Australia, may be severely disadvantaged with respect to survival and reproductive success.

Choosing a wintering site is an important task for migratory birds, as birds in favourable locations have higher survival and possibly even a better breeding success in the following summer. By comparing three different wintering habitats at different latitudes using physiological tools (stress biomarkers and parasite infection rates), we find further support for the high benefits of wintering at high latitudes (Chapter 5). Birds wintering in South Australia and Tasmania (35-40°S) were found to have better health indices than birds wintering in Broome, north Western Australia (18°S). The use of physiological tools provide a useful means for assessing habitat quality in terms of the physiological effects on the animal itself, and non-invasive tools are increasingly being used for this task. A validation study presented in this thesis (Chapter 6) confirms the validity of using feathers to measure stress hormones in birds. However, it also emphasises the need for caution in the interpretation of such data, in part because of individual variation.
Chapter 1

General Introduction: Challenges within the annual cycle of long-distance migratory waders from the East Asian-Australasian Flyway

Yaara Aharon-Rotman
In the Middle-Ages, the disappearance of some birds (e.g. swallows and martins) during winter was speculated to be because the birds were hibernating at the bottom of lakes until reappearing in spring (Magnus 1555). We have come very far with understanding that these departures represented migratory processes, but there are still many gaps in our knowledge of seasonal bird movements (Fryxell et al. 2011).

Fryxell et al. (2011) described migration as “…fine art, something that everyone knows about, a subject on which everyone has a strong opinion, yet nobody can precisely define”. Indeed, although scientists have suggested various definitions of migration (Baker 1978, Kennedy 1985, Dingle and Drake 2007, Salewski and Bruderer 2007), there are some complex and variable movements, notably in the southern hemisphere, which may not involve return trips within an annual cycle, but instead may be irregularly scheduled to coincide with unpredictable weather events with a time scale often more than a year (Dingle 2008). Some species also regularly engage in long-distance return trips that are not migratory movements, but represent multi-day foraging trips (e.g., albatrosses and petrels). Relative to the abundance of information regarding birds in the northern hemisphere, little is known about the migratory habits of birds in the southern hemisphere, as most of the avian studies in this part of the world have focused on sedentary rather than migratory species (Clarke 1997). We are therefore still at the stage of describing patterns of migration in the southern hemisphere, rather than proposing evolutionary hypotheses to explain their existence (Dingle 2008).

Nowadays, much of our understanding of migration is based on new tracking technology, enabling us to follow migrants for long distances in water, on land and in the air. Satellite-based tracking devices have been widely used for migratory birds. However, the increasingly shrinking weight and size of geolocation dataloggers has led to a boost in data gathering, because, despite their reduced spatial accuracy, geolocators have made research possible on limited budgets and in species as small as 15g (Bridge et al. 2011). Geolocators have been successfully used on many migrant species including the bar-tailed godwit *Limosa lapponica baueri* in New Zealand, revealing their amazing non-stop southward migration across the pacific (Gill et al. 2009). They have also been deployed on the red knot *Calidris canutus rufa* in Delaware Bay (Niles et al. 2010), redshanks *Tringa Totanus* in Sweden and ruddy turnstones *Arenaria interpres* in Australia (Minton et al. 2010).
Migration is a complex behaviour, arising from interactions between individuals, their genes, and their environment (Cresswell et al. 2011). Migration has evolved to take advantage of temporal variations in the spatial distribution of resources (food and nest sites) and danger (predators and diseases) (e.g. Salewski and Bruderer 2007, Louchart 2008), while also being influenced by the changes in specific resource needs of the animals during the different life-history stages (Cresswell et al. 2011). For example, for many Arctic-breeding migrants, cold and food shortage are the drivers in moving south before winter sets in in the northern hemisphere. The advantage they gain by reducing their thermoregulatory costs by overwintering close to the equator outweighs the costs of flying to these locations. Still, some migrants remain in north-temperate latitudes over the winter or even fly extra distances beyond the equator to winter in south-temperate latitudes during the austral spring and summer (Newton 2003, Dingle 2008). Conversely, the migration back to the Arctic breeding grounds is thought to be linked to the locally high seasonal productivity, low predation pressure and the relative absence of pathogens.

Migratory species are thought to have an energetically expensive existence, as their annual flights often involve crossing long-distances and many barriers. A typical feature of migratory animals is the build-up of fuel stores in preparation for migration. Indeed, it could be argued that, rather than flying, foraging is actually the dominant behaviour during the migratory period. These fuel stores importantly consist of energy-dense fat, but protein is also an important component (Klaassen 1996). Still, although some migrants may nearly triple in weight before departing on a migratory flight (Lindström et al. 2011), most migrations cannot be completed in one flight or on a single fuel store. Most migrations thus involve the use of stop-over sites to recover from the effort and refuel the depleted energy stores. Thus, besides migratory preparations at the sites of origin (prior to southward and northward migrations), birds also need to fuel at one or more stop-over sites along the route.

Many of the world’s migration systems are threatened (Wilcove and Wikelski 2008). Consequently, many migratory species are facing major challenges, mainly caused by global change processes. While it would appear that a migrant’s reliance on multiple habitats is potentially advantageous in evading particular deteriorating environments, the probability of a site to be affected by environmental changes increases with the number of sites used (“multiple jeopardy”), which actually increases their vulnerability (Newton 2004).
Besides the importance of protecting declining migratory populations from a species conservation consideration, it is important to recognise that migratory species have great effects on the ecosystems of which they form a part (Bauer and Hoye 2014). These can be broadly divided into “trophic” and “transport” effects (Holdo et al. 2011). Trophic effects are a direct result of food consumption. For example, curlew sandpipers *Calidris ferruginea* and red knots *C. canutus* wintering in South Africa were found to have a significant impact on the littoral benthos, consuming large amounts of worms and other small prey items (Blomqvist et al. 2002). By contrast, the transport effects are indirect, and are the result of migrants acting as dispersal tools for various organisms, including diseases, nutrients and energy (Holdo et al. 2011). As a more extreme case of migrants acting as a transport vehicle, free living organisms such as aquatic snails were recently found to hitch hike in the guts of waterbirds and travel from one water body to the next (Van Leeuwen et al. 2012). The potential impact that reductions in migratory birds would have on such effects reinforces the importance of conservation efforts to protect their numbers.

Migratory shorebird populations (waders) are also under threat and declining (IWSG 2003). Migratory shorebirds use distant habitats and cover the distance between these habitats in long flights that often span across continents and even hemispheres (Webster et al. 2002). Conservation strategies for intercontinental migrants must recognise migratory connectivity and carry-over effects (Runge et al. 2014) that is, events that happen at one place or in one season might influence what happens in subsequent places/seasons (Norris 2005). Migratory shorebirds are known to have well-developed cognitive abilities (Mettke-Hofmann and Greenberg 2005). However, as they are long-lived species, they may lack sufficient capacity to evolve at a rate matching the rates of the rapid global environmental changes, making them particularly vulnerable (Rosenheim and Tabashnik 1991).

The global decline of many wader populations is mostly thought to be due to degradation and loss of stop-over and wintering habitats (Melville 1996, Barter 2002, Baker et al. 2004, Morrison et al. 2004, Burton et al. 2006, Newton 2006, Fernandez and Lank 2008, Clemens et al. 2010, Rogers et al. 2010). Yet, changing conditions at the breeding grounds have also been suggested to play a role in their demise (Newton 2004, Koivula et al. 2008, Fraser et al. 2013). Whatever the precise location of these declines, global change processes are widely considered to stand at their base, the declines being attributed to different direct and indirect anthropogenic effects, including sea level rise.
There is particular concern for declining shorebird populations along the East Asian-Australasian flyway (EAAF) (IWSG 2003, Amano et al. 2010, Cox 2010, Wilson et al. 2011), where numbers of some migratory species have declined dramatically over the past few decades (R. Fuller, pers. comm, MacKinnon et al. 2012). Habitat loss along this flyway has been suggested as the prime reason behind these declines (Barter 2002, Barter et al. 2003, van de Kam et al. 2010). The Yellow Sea is considered to be one of the major stop-over sites for waders migrating along the EAAF and is importantly suffering from habitat loss. It is located between the Korean peninsula in the east and the Chinese coast to the west, and is one of the biggest intertidal areas globally, with an estimated one million hectares of intertidal mudflat (Kam et al. 2004). Two major rivers flow into the sea: the Huang He (the Yellow River) and Chang Jiang (Yangtze River). About two million shorebirds (which are roughly 40% of all birds in the EAAF) use the Yellow Sea area during northward migration in March till May (Barter 2002). Thirty-seven sites around the Yellow Sea are identified as internationally important. Data from North Korea is limited to the Mundok Migratory Bird Wetland Reserve (Hua et al. 2015).

As a result of rapid development and high population density, 51% of all coastal wetlands in China, the second largest economy in the world, have been lost (MacKinnon et al. 2012). The “new Great Wall”, a seawall of thousands of kilometres built to create extra land for the rapidly growing urbanization and economy caused a dramatic decline in biodiversity and threaten the ecology of the Chinese coastal area (Ma et al. 2014). The effect is shown notably in the Yellow Sea, where 28% of tidal flats existing in the 1980s had disappeared by the late 2000s (Murray et al. 2014). The two main rivers flowing to the Yellow sea are still significantly changing and have greatly reduced their sediment input. High levels of pollution and human activities, including unsustainable harvesting, have led to a decrease in benthic productivity, resulting in reduced food supply for shorebirds (Barter 2002). Not only are birds negatively affected by the serious ecological condition of the Yellow Sea, declines are also observed in other taxa such as marine mammals (Ross et al. 2010), green turtles Chelonia mydas (Song et al. 2002) and important seaweed beds and sea grasses (Short et al. 2011). These adverse impacts demand that drastic changes in agricultural and urban planning must take place if the ecological functioning of the Yellow Sea is to be restored.

Considering the rapid decline in wader numbers and the rapid ongoing environmental changes along the EAAF, urgent actions must be taken. The large geographic range across which migratory behaviour takes place does not allow for a traditional conservation approach
focusing on sites in isolation. Focusing on a single isolated site rather than the chain of sites that form a migratory route will not produce an optimal protection strategy. More advanced methodologies are needed, leading to informed conservation prioritisation and actions that target key stopover sites along the migratory itineraries, and thus are the most critical links in the migratory chain (Runge et al. 2014).

Global change processes are not exclusively restricted to Asia and areas like the Yellow Sea. Many authorities do not employ the precautionary principle when it comes to nature conservation, but instead require firm evidence before they are convinced to make decisions that are not justified simply on economic grounds. In the case of the EAAF shorebirds, as well as other migratory systems, a holistic approach is needed which includes identification of the main factors most likely to constrain migrants along their entire migratory chain.

The annual cycle of migrants can be divided into four stages: **Breeding, migration** to the non-breeding sites, **wintering** and migration back to the breeding sites. The aim of my thesis is to shed more light on some of the factors associated with global change processes that are considered to explain the decline of waders along the EAAF. Each of my chapters will address one or more stages in the annual cycle of migrants, aiming to identify where changes may have adversely affected migratory shorebirds.

I have mainly focused on ruddy turnstones (chapters 4 and 5) because the advances in tracking technology using geolocators and their site-faithfulness to wintering grounds has allowed high recapture rates. This has enabled extensive tracking of individuals along the migration route over recent years, resulting in enhanced knowledge on the migratory behaviour of this species. Tracking results for ruddy turnstones in Australia has revealed a previously unknown southward migration route through the Pacific in addition to the known route along the east coast of Asia, which is taken by the majority of the population. This fascinating discovery further encouraged us to explore and focus our study on this remarkable species, while generalizing our results to other migrants with comparable ecological characteristics where possible.

**Breeding**

I start the thesis where the life of every shorebird begins, on the breeding ground. Although under current conditions migration along the EAAF may be an increasingly arduous enterprise, the advantage of migrants is the ability to move and raise offspring in favourable
faraway places as the high Arctic, where food is temporarily abundant, days are long (Schekkerman et al. 2003), and predation (McKinnon et al. 2010) and disease (Mendes et al. 2005) risks are low.

However, in winter the Arctic environment is harsh and thus does not allow birds to stay year round. The short summer limits food availability to only few months and requires suitable timing of arrival and departure, before temperatures start to drop again (Drent et al. 2006, Hedenström 2008). Early arrival is advantageous as birds can (i) choose better nesting territories, (ii) choose better feeding sites to prepare for egg laying, (iii) choose a better mate (Tulp 2007) and (iv) enjoy a longer breeding season (Newton 2008). However, arriving too early may also have negative consequences (Fig. 1). Firstly, early in the season temperatures are still low and will enforce increased energy expenditure for thermoregulation. Secondly, food is still not highly abundant and the risk of body mass loss and even starvation is higher. Thirdly, as snow just starts to melt, nesting in one of the few and small snow-free patches may result in higher predation pressure (Byrkjedal 1980).

Climate change effects are notably felt in the high-Arctic (Arctic-amplification; Pithan and Mauritsen 2014) and it is also the place where, possibly paradoxically, many toxins accumulate because of climate-related factors. Practically all pollutants in the Arctic originate from more southern latitudes and are transported to the Arctic by winds and ocean currents (Law and Stohl 2007). As a consequence of bioaccumulation, many persistent organic pollutants (POPs, such as PCBs, DDTs, and PBDEs) are found in Arctic biota and humans (Letcher et al. 2010, Erikstad et al. 2013). There has been little experimental work on the toxic effects of POPs or trace elements in shorebirds, but effects on mortality (Braune and Noble 2009) and reproductive success (Hoffman et al. 1996) have been recorded. This warrants a closer look at patterns of breeding success over time in migratory shorebirds of the EAAF.

![Figure 1: Seasonal variation in snow cover, temperatures and food availability during different stages of the Arctic breeding season. Eggs are laid when snow starts to melt, ensuring that chicks will hatch around the peak in temperature and arthropod abundance. Horizontal bars indicate periods of appearance of adults and chicks in the area (from (Tulp 2007)).](image-url)
Besides studying long-term trends, inter-annual variations may also be of particular interest for study. Three main factors have been suggested to explain inter-annual variation in breeding success of waders in the Arctic. The first suggests that cold summers inhibit breeding success, as low temperatures have a negative effect on fitness. Indeed, positive correlations were found between summer temperatures and breeding success of sharp-tailed sandpipers *Calidris acuminata*, red necked stints *C. ruficollis*, curlew sandpipers and ruddy turnstones in the EAAF (Soloviev et al. 2006), as well as of little stints *C. minuta* and dunlins *C. alpina* in the East Atlantic Flyway (Tulp 2007). The second factor suggests a link between breeding success and the timing of snow melt. A prolonged period of snow cover limits the availability of pre-nesting feeding areas, reduces availability of nesting sites and increases predation risk while incubating (Byrkjedal 1980, Madsen et al. 2007). Thus, ultimately, late snowmelt has negative consequences on fitness and nest success. The third factor suggests that Arctic lemmings are responsible for fluctuations in the success of locally breeding geese and waders according to the *Alternative Prey Hypothesis*. Lemming numbers typically show three to four-year cycles and are potentially regulated by food-plant availability (bottom up) and their predators, such as Arctic fox *Vulpes lagopus*, stoat *Mustela erminea* and snowy owl *Bubo scandiacus* (top-down). The *Alternative Prey Hypothesis* suggests that predators, after having increased in numbers during a lemming peak, will switch to chicks and eggs of waders and geese when lemmings are scarce, lowering these birds’ breeding success (Summers 1986, Dhondt 1987, Béty et al. 2001, Kausrud et al. 2008, Lecomte et al. 2008). In light of the increased evidence that lemming cycles have started to change over the last two decades, in Chapter 2 I examine the effect of lemming abundance, as well as temperature in the Arctic summer on the breeding success of high Arctic nesting shorebirds.

**Migration - strategies**

There are different ways in which birds can go about spending the energy that has been accumulated during pre-migratory fuelling. Basically three different strategies have been proposed which vary in their flight distances and thus energy requirements: hop, skip and jump; from the less energetically demanding strategy to hop short distances, to the challenging strategy of accumulating large stores of extra fuel to jump long distances between sites (Piersma 1987). Under the time-minimisation hypothesis (Alerstam and Lindström 1990, Lindström and Alerstam 1992), the two extremes, hopping or jumping, will evolve if the potential for fuelling is evenly or irregularly distributed along a flyway,
respectively. An example of such a long distance “jumper” is a subspecies of Bar-tailed Godwit (Limosa lapponica baueri), which makes a remarkable non-stop flight of up to 11,680 km from the Alaskan Arctic to the wintering grounds in New Zealand (Gill et al. 2009). Of course there is a trade-off between these strategies, and although “jumping” long distances requires carrying great amounts of fuel to survive the journey, the “hoppers” have settling costs every time they stop to refuel, as they need to find food and safe places to stay (Alerstam and Lindström 1990).

In chapter 3 we suggest an explanation for the migration strategy that many cross-equatorial migrants use, based on spatial variation in fuel deposition rates. These strategies, which are characterised by long migration legs (jumps) when leaving staging sites at high (absolute) latitude followed by shorter hops/skips towards the final destination, are probably a result of a latitudinal trend in fuel deposition rate. Under the premise of such a latitudinal trend in fuel deposition rate, the observed characteristic migratory strategy in cross-equatorial migrants is congruent with predictions from the time-minimisation hypothesis.

**Migration – deteriorating stop-over sites**

Sites where migrating birds stop to rest, drink and feed are essential for them to recuperate and replenish body stores in preparation for the next migratory leg. If migrants are not able to satisfy their energetic requirements, this can have direct and immediate consequences for survival but also knock-on effects, affecting survival and reproduction at a later stage (“carry over effects”) (Gill et al. 2001, Warnock 2010).

Habitat loss is one of the greatest threats to bird biodiversity worldwide, and mostly attributed to different human activities and, notably in the case of waders, sea level rise (Fernandez and Lank 2008). So far, 40% of the most productive land worldwide is cultivated by man, emphasising the immense conflict between man and nature, including migrants (Klaassen et al. 2012). As mentioned earlier, the phenomenon is also common in the coastal areas of East Asia (MacKinnon et al. 2012, Murray et al. 2014), putting shorebirds of the EAAF under threat, as they intensively use these areas for fuelling during migration (Weber et al. 1999, Barter 2002, Clemens et al. 2010).

The effect of habitat loss on populations may be disproportional to the loss of habitat, as some species are highly specialized on specific food sources. For example, only 20km of the coast in Bohai Bay supports over 45% of the flyway population of red knots *Calidris canutus*
piersmai and C. c. rogersi during northward migration (Rogers et al. 2010). Similarly, a large portion of the population of bar-tailed godwits depends on a single site, the Yalu Jiang (Barter and Riegen 2004) during their annual migration between their wintering sites in Australia and their breeding grounds in the high Arctic. Recent counts from 2010-2012 estimated that this site supports an annual average of over 68,000 individuals, about half of the whole flyway population (Choi et al. 2015). Clearly, loss of these relatively small but highly preferred sites may result in significant population declines.

To cope with sites where conditions are deteriorating (e.g. high competition, predation risk) migratory birds might theoretically change their routes, potentially without major fitness loss (Burton et al. 2006, Klaassen et al. 2006, Bauer et al. 2010). However, not all birds may be capable of such change, as they may be constrained by extreme site fidelity (Burton et al. 2006). This trait is highly conserved in many migratory shorebirds to the main non-breeding ground (e.g. Warnock and Takekawa 1996, Leyrer et al. 2006, Minton et al. 2006, Mittelhauser et al. 2012) as well as to stop-over sites (et al. 2015).

In light of the current global changes, in chapter 4 we assess the consequences of habitat loss and degradation of sites along the EAAF in terms of migratory behaviour, survival and reproductive success assuming that shorebirds, and in this case more specifically ruddy turnstones, are capable of making optimal decisions in the face of environmental changes along their flyway. To this end, we used Stochastic Dynamic Programming (SDP) that can be applied to address responses of migratory birds that use multiple distinct stop-over sites to environmental changes (Weber et al. 1998, Weber et al. 1999, Klaassen et al. 2006, Klaassen et al. 2008, Bauer et al. 2010).

**Wintering**

Migratory shorebirds spend about half of the year on the main non-breeding ground (‘wintering’ ground), where many of them moult and prepare for the next long flight and breeding season. To allow for long-distance migration, appropriate preparations are essential, including behavioural, physiological and morphological adjustments to increase flight performance and maximize energy gain from foraging (Piersma and Lindström 1997, Piersma and Gill 1998, Klaassen et al. 2012).

Large-scale variation in conditions across sites results in different energy costs and benefits across the migratory range (Quaintenne et al. 2011). Carry-over effects may play an
important role in population dynamics (Norris 2005), with those individuals selecting pre-migratory staging areas at better sites being more likely to attain higher body condition prior to departure and thus better survival and breeding success (Marra et al. 1998, Smith and Moore 2003, Alves et al. 2013). Traditional methods for estimating habitat quality using population-level fitness estimations and direct measurements of habitat features may fail to establish a link between population dynamics and habitat characteristics (Homyack 2010). An alternative approach is to evaluate the physiological responses of individuals and populations to varying environments (“conservation physiology”; Wikelski & Cook 2006) by quantifying constraints and stressors that may affect individual fitness using vitality proxies such as immunological, endocrinological and biochemical biomarkers (Albano 2012, Klaassen et al. 2012).

In Chapter 5 we use vitality measures to evaluate the quality of three wintering habitats in Australia and suggest a reason as to why some populations fly all the way to winter in the southern part of Australia rather than stay in the tropics. One of the biomarkers we used was the corticosterone content of feathers (CORT\textsubscript{f}), which provides an integrated estimate of stress history while birds moult their feathers at wintering sites.

Clearly the metabolic demands or stress on migratory birds change continuously throughout the migratory period. CORT plays a crucial role in helping birds to cope with these stressors and to restore their homeostasis. The stress-response (Fig. 2) is triggered by activation of the hypothalamic-pituitary-adrenal (HPA) axis (Sheriff et al. 2011). When CORT levels rise, animals redirect their behaviours and resources towards self-maintenance activities and may then reach an emergency state if initial responses fail to overcome the stress (Bortolotti et al. 2008; Charmandari, Tsigos & Chrousos 2005).

Figure 2: Acute and chronic stress implications under stress response (Boonstra et al. 1998)
acute environmental challenges. Under acute stress the HPA system is dynamically regulated by negative-feedback mechanisms, which restore the system to its pre-stress state. However, when exposure to stressors is chronic and at high level, feedback signals are weakened and the system has difficulties maintaining homeostasis (Fig. 2) (Boonstra et al. 1998). Exposure to chronic stress has detrimental health consequences in regard to decreases in body condition, growth, reproduction and survival (Blas et al. 2007; Charmandari, Tsigos & Chrousos 2005), and might also adversely affect fuelling rates. In addition, prolonged exposure to a variety of stressors can suppress immune function, resulting in higher susceptibility to infections (Gleeson 2007).

Recently, the use of feather CORT as a stress biomarker has gained popularity (Bortolotti et al. 2008, Bortolotti et al. 2009, Lattin et al. 2011). Nevertheless, the reliability of this method to reflect stress status during moult has seldom been validated (Lattin et al. 2011, Fairhurst et al. 2013). We therefore validated the use of feathers to estimate CORT levels in birds using experimental manipulation of plasma CORT levels in house sparrows, Passer domesticus, the findings of which we report in Chapter 6.
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Loss of periodicity in breeding success of waders links to changes in lemming cycles in Arctic ecosystems

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Oikos (2014)
Abstract

Lemming population cycles in the Arctic have an important impact on the Arctic food web, indirectly also affecting breeding success in Arctic-nesting birds through shared predators.

Over the last two decades lemming cycles have changed in amplitude and even disappeared in parts of the Arctic. To examine the large scale effect of these recent changes we re-analysed published data from the East Atlantic Flyway (EAF), where a relationship between lemming cycles and wader breeding success was earlier found, and new data on breeding success of waders in the East Asian- Australasian Flyway (EAAF).

We found that (1) any long-term periodicities in wader breeding success existed only until the year 2000 in the EAAF and until the 1980s in the EAF; (2) studying these patterns at a smaller spatial scale, where the Siberian-Alaskan breeding grounds were divided into five geographical units largely based on landscape features, breeding success of waders from the EAAF was not correlated to an index of predation pressure, but positively correlated to Arctic summer temperatures in some species.

We argue that fading out of lemming cycles in some parts of the Arctic is responsible for faltering periodicity in wader breeding success along both flyways. These changed conditions have not yet resulted in any marked changing trends in breeding success across years, and declining numbers of waders along the EAAF are therefore more likely a result of changing conditions at stop-over and wintering sites.
Introduction

Several studies found breeding success of Arctic migratory waders (shorebirds) and geese to be linked to variation in lemming abundance (e.g. Bêty et al. 2001, Blomqvist et al. 2002, Martin and Baird 1988, Summers and Underhill 1987). This argument relies on the Alternative Prey Hypothesis (Roselaar 1979, Summers 1986), which suggests that lemming cycles in the Arctic are responsible for fluctuations in the success of locally breeding geese and waders. Lemming numbers typically show three to four-year cycles and are potentially regulated by food-plant availability (bottom up) and their predators, such as Arctic fox *Vulpes lagopus*, stoat *Mustela erminea* and snowy owl *Bubo scandiacus* (top-down). Whether lemming cycles are bottom up or top down controlled is in dispute (e.g. Andersson and Erlinge 1977, Gilg et al. 2003, Krebs 2011, Turchin et al. 2000). The Alternative Prey Hypothesis suggests that predators, after having increased in numbers during a lemming peak, will switch to chicks and eggs of waders and geese when lemmings are scarce, lowering these birds’ breeding success (Bêty et al. 2001, Dhondt 1987, Kausrud et al. 2008, Lecomte et al. 2008, Summers 1986).

Periodicity in lemming abundance is shown in parts of the Arctic (Ims et al. 2013, Reid et al. 2013). However, cycles have changed, and even disappeared in some parts over the past two decades. Collapsing cycles were first reported from Fennoscandia (Kausrud et al. 2008), followed by reports on their disappearance from areas in Greenland (Gilg et al. 2009) and Yamal Peninsula, NW Siberia (S.Mechnikova, unpublished), whereas on Wrangel Island, Russia, a change in the length of cycles was reported (Menyushina et al. 2012). Although lemmings may have drifted in and out of cycles previously (Ims et al. 2008), similarly changing population cycles in other Arctic animal species (e.g. voles, black grouse *Tetrao tetrix* and forest insects) suggests the involvement of a common, large-scale effect, such as climate change (Ims et al. 2008). In the case of lemmings, changing snow conditions in winter are assumed to be key to these population dynamic changes (Ims et al. 2011, Kausrud et al. 2008), with cascading knock-on effects on linked species (Schmidt et al. 2012). Nonetheless, evidence of returning vole cycles in south Finland in the early-2000s, after being absent for five years, (Brommer et al. 2010) and variation in patterns of periodicity between climatologically different regions in Finland (Korpela et al. 2013) have been suggested to refute this theory (Brommer et al. 2010).
Worldwide, waders are of particular conservation concern owing to their long distance migration, low reproductive rate and dependence on specific habitats that are globally deteriorating (Cox 2010, Fernandez and Lank 2008). Degradation and loss of stopover and wintering habitats are widely considered to be the cause of their generally declining numbers (e.g. Fernandez and Lank 2008, Norris 2005, Rogers et al. 2010). Yet, climatic changes at the breeding grounds, and the interaction of climate and existing predator-prey cycles, is increasingly being considered to potentially play a role (Fraser et al. 2013, Newton 2006, Zöckler et al. 2000). Given the rapid economic developments along the East Asian-Australasian Flyway (EAAF, Fig. 1A) and the changing climatic and biotic conditions on the Arctic breeding grounds, there is notably great conservation concern for the 8 million and declining waders using this route (Bamford et al. 2008, Cooper et al. 2012, Wilson et al. 2011).

We investigated whether (i) lemming cycles on the breeding grounds, or the lack thereof, are reflected in the periodicity in breeding success of waders on the East Asian-Australasian Flyway (EAAF) and (ii) whether predation pressure together with average summer temperatures across five geographic units within the Siberian-Alaskan breeding area may explain inter-annual variation in breeding success of waders from the EAAF. Along with the EAAF data we also re-analysed data from the East Atlantic Flyway (EAF, Fig. 1A), where a strong effect of lemming cycles on breeding success was previously shown (Blomqvist et al. 2002), to evaluate any changes in this effect over time.

Materials and Methods

Breeding success of waders
Published juvenile percentages as observed on the wintering grounds were used as estimators of breeding success of Arctic-breeding waders. Despite its limitations (Clark et al. 2004, McCaffery et al. 2006, Rogers et al. 2006), this is currently the most practical method for estimating breeding success especially in long-term monitoring of long-distance migrants (Minton 2003). Data was available in the EAAF for 1978-2013 in south east Australia (SEA) for ruddy turnstone *Arenaria interpres*, red-necked stint *Calidris ruficollis*, curlew sandpiper *C. ferruginea*, sharp-tailed sandpiper *C. acuminata*, red knot *C. canutus* and bar-tailed godwit *Limosa lapponica* (Minton et al. 2011a, Minton et al. 2005, Straw 2003). For Broome and Eighty-mile Beach, north Western Australia (NWA), similar data was available for 1998-2013 for red-necked stint, curlew sandpiper and bar-tailed godwit (Minton et al. 2011a). We
refer to the Australian and African non-breeding grounds (belonging to the EAAF and EAF, respectively) as “wintering” areas throughout this paper, as these sites are used during the northern hemispheric winter. We chose only wader species for which breeding areas coincide with lemming abundance areas. These species breed in a vast area in the Russian-Alaskan tundra (especially curlew sandpiper and red-necked stint). However, we identified core breeding areas based on recoveries and flag-sightings. This data was obtained from Lappo et al. (2012) and Minton et al. (2011b) and matched with the five geographic regions identified below.

For the EAF, juvenile percentages of three wader species wintering in South Africa (curlew sandpiper, red knot and sanderling) were obtained from Blomqvist et al. (2002) and include the years 1969-1986. For curlew sandpiper additional data from 1947-2005 from an autumn stop-over site in Ottenby, southern Sweden, was used (Barshep 2011).

**Identification of geographic regions**

Lemming population cycles tend to be synchronous across regions with relatively uniform landscape structure (Angerbjorn et al. 2001, Chernyavsky and Lazutkin 2004) (but see Henden et al. (2009) for periods where populations were less synchronous in Fennoscandia). Accordingly, we divided the tundra zone of eastern Siberia and northwestern north America into five geographical regions: *East Taimyr* (from the mouth of the Verkhnyaya Taimyra River in the central Taimyr Peninsula to the Anabar River), *Lena Delta* (area between the Anabar and Yana Rivers), *East-Siberian Plain* (from the Yana to Kolyma River), *Chukotka* (from Chaun Bay to the Bering Strait) and *Alaska* (Fig. 1B). For each we obtained lemming abundance by combining information from observation points within each region, as described below. Most of these regions have different landscapes: Gently sloping hilly landscape predominates at Taimyr, flat marshy tundra on the East-Siberian plains and mountains in Chukotka. The Lena Delta with adjacent areas as well as Alaska have a more complex composition of landscapes with huge deltas, hilly areas and mountain ranges.

In some cases, clear natural borders exist between the neighbouring regions – Alaska and Chukotka are separated by the Bering Strait, the western edge of the Chukotka region generally coincides with the border between the mountainous and flat plain landscapes. Borders between the other areas are more arbitrary but have been chosen such as to best separate the various wader populations. Also the western border of the East Taimyr region is
arbitrarily chosen as 100°E, but it also coincides with the westernmost area where shorebirds of the EAAF are known to breed.

**Figure 1:** (A) Descriptions of the East Asian-Australasian Flyway (EAAF, light grey) and the East Atlantic Flyway (EAF, dark grey) for waders discussed in this paper. (B) The five geographic regions identified within the Siberian-Alaskan high-Arctic breeding range of waders from the EAAF. For each region the species of waders that find their core breeding range within that region are indicated. For Bar-tailed Godwit, which forms two different populations, the major non-breeding ground in Australia is mentioned in brackets (SEA for south east Australia and NWA for north Western Australia).

<table>
<thead>
<tr>
<th>East Taimyr</th>
<th>Chukotka</th>
<th>Alaska</th>
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<tr>
<td>Red-necked Stint</td>
<td>Red-necked Stint</td>
<td>Bar-tailed Godwit</td>
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<tr>
<td>Curlew Sandpiper</td>
<td>Red Knot</td>
<td>(SEA)</td>
</tr>
</tbody>
</table>

_East Siberian Plains_  
Ruddy Turnstone  
Sharp-tailed Sandpiper  
Bar-tailed Godwit (NWA)  
Curlew Sandpiper

_Lena Delta_  
Red-necked Stint  
Curlew Sandpiper

**Lemming abundance and predation index**

For the EAAF analysis most lemming abundance data were collected in the framework of the Arctic Birds Breeding Conditions Survey (ABBCS), covering the entire Arctic breeding area of the waders within the EAAF over the period 1989-2013 (all original data available at http://www.arcticbirds.net). Data for 1978-1988 and additional information to complement the data from ABBCS was obtained from the literature (Chernyavsky and Lazutkin 1999,
All lemming abundance data were indexed on a scale from 1-3, corresponding to low, average and high, although intermediate values could be obtained when local scores within a region were averaged (Appendix S1). When quantitative information (mostly trapping data) was available, the three levels corresponded to 0-3, 4-10 and over 11 specimens captured per 100 trap-days. This ranking system largely follows the one used by Kokorev and Kuksov (2002), who additionally used a “very low” and “very high” category which we combined with “low” and “high”, respectively. When quantitative information was lacking assignment of rank was based on the mutual agreement of two experts (MS and PT), who evaluated qualitative information available in breeding conditions reports in the ABBCS database. For the EAF analysis, lemming abundance data for the breeding grounds on Taimyr Peninsula covering the period from 1947 until 1995 were obtained from Blomqvist et al. (2002) and for 1996-2010 from the ABBCS.

Predation index \( P \) was calculated following Blomqvist et al. (2002) using the lemming abundance index (ranging from 1-3) in the previous \( (X_{t-1}) \) and the present year \( (X_t) \):

\[
P = \frac{(X_{t-1} - X_t + 3)}{2}
\]

This index (ranging from 0.5-2.5) is based on the assumption that in years with low lemming abundance following lemming peaks, predators will be abundant and divert their predation from lemmings to bird eggs and chicks.

**Temperatures on the EAAF breeding grounds**

Daily average temperature data (number of readings per day span from six in earlier years to 24 in recent years) from 15 May to 31 July over the years 1978-2012 from all available weather stations in the area from east Taimyr to Alaska (Global Surface Summary of the Day at NOAA/National Climatic Data Centre [ftp://ftp.ncdc.noaa.gov/pub/data/gsod]) were used to create daily air temperature surface maps using the Manifold 8 GIS system. The interpolation algorithm was gravity (Manifold's implementation of inverse distance interpolator), with a search radius of 500 km and using a maximum of ten stations. Next, the surface maps were overlayed with the breeding ranges of each wader species from the EAAF and surface values averaged within these ranges, resulting in a daily temperature value for
each species. Daily values were subsequently averaged to obtain the mean for the second half of May and monthly means for June and July.

**Statistical analyses**

For all wader populations along both the EAAF and the EAF, except for red knot from SEA because of data shortage, we used autocorrelation and partial autocorrelation functions (ACF and PACF, respectively) to test for periodicity in breeding success (i.e. juvenile percentages), using the *acf* function in the *stats* package of R (R Core Team 2014). ACF is used to describe the correlation of a variable with itself at two points in a time series. PACF describes the correlation between a variable and itself in two different time points, once correlation between all successive lags are controlled, and is used to determine which time lags are involved in the generation of the cycles observed in the ACF (Crawley 2012b). To investigate a potential link between periodicity in breeding success and lemming cycles, a similar analysis was also performed on lemming abundance indices for the geographical areas mentioned above.

These correlation techniques assume that the statistical properties of the time series are stationary (i.e. consistent over time), an assumption which is often violated in ecological systems (Cazelles et al. 2008). To reveal whether periodicity in breeding success changed over time for wader populations along both the EAAF and the EAF we used two different approaches: (1) a moving window of 20 years, starting in the first year of the time series, to test the changes in the autocorrelation over time and (2) wavelet analysis. Wavelet analysis has been shown to be optimal for detecting changes in ecological time series, as it can deal with aperiodic, noisy and varying signals (Torrence and Compo 1998). However, long data sets are required for a reliable wavelet analysis, which we thus limited to species with data sets exceeding 30 years in length. Data was sufficiently long in three species from the EAAF (curlew sandpiper, sharp-tailed sandpiper and red-necked stint, all from SEA) and in only one species from the EAF (curlew sandpiper from Sweden). Wavelet analysis was performed using the *wt* function in the *biwavelet* package in R, using all default settings, including “morlet” as the mother wavelet (Gouhier 2004). A regular $\chi^2$ test was used for significance testing. Periodicity estimates at the start and end of time series are fraught with error, which is recognised by *biwavelet* calculating a “cone of influence” for each wavelet analysis identifying the region in a time series in which edge effects become important.
A similar analysis was also performed on the lemming indices in three breeding areas for waders of the EAAF for which the time series were sufficiently long (East Taimyr, Chukotka and East Siberian plains) as well as Taimyr Peninsula, the breeding area of curlew sandpiper from the EAF. Wavelet analysis requires uninterrupted data series, yet, for two years in Chukotka (1995 and 1999), two years in east Taimyr (1982 and 1984) and three years in the East Siberian plains (1985, 2002 and 2003) gaps in the data series occurred. In these cases we trialed all possible combinations of lemming indices (1,2 and 3) across the missing years to test, and in all three cases indeed confirm, the robustness of the trend. We therefore completed these missing values with an average index (2).

Dispersion parameter in the breeding success data ($\phi = \text{deviance divided by the degrees of freedom of the residuals}$) was high in all cases due to many low juvenile percentages. We therefore used a quasi-binomial logistic model (Crawley 2012a) when analysing breeding success in relation to temperature and predation index. For each bird population along the EAAF we ran a model with the annual proportions of juveniles from wintering wader populations, weighted for total sample size, as the dependant variable, predation index as categorical predictor, and average temperatures in late May ($T_{\text{may}}$, °C), June ($T_{\text{jun}}$, °C) and July ($T_{\text{july}}$, °C) as continuous predictors. We also ran a model for curlew sandpiper from Sweden (EAF) with predation index as categorical variable. All predictor values in the EAAF models were standardized (i.e. Z-transformed), to ease assessment of their contribution to the ultimate model. We limited core breeding area for all populations except curlew sandpiper and red-necked stint to one of the five geographic regions identified above (Fig. 1B). For curlew sandpiper and red-necked stint predation index was averaged across the various geographic regions where they breed prior to standardization. Although weakening the correlation, this step was essential due to the vast range of breeding locations of these species across the Russian tundra. For each population, model selection was achieved through ANOVA F-tests (as quasi binomial models do not provide log(likelihood) values for the calculation of AIC). Subsequently, for each parameter in the final model we calculated the odds ratio (OR), which is the increase in breeding success for every unit of increase in the predictor variable (while holding all the other variables in the model at a fixed value). An OR>1 indicates a positive, whereas an OR<1 indicates a negative effect of the predictor variable.

All statistical analyses were conducted using R version 3.0.3 (R Development Core Team, 2014). Significance level of all statistical tests was set at $p=0.05$. 

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Results

Periodicity in breeding success

Autocorrelation in the juvenile percentages data on waders from both EAF and EAAF resulted in significant three-year periodicity, confirmed by partial autocorrelation, for red-necked stint from SEA only (Appendix S2).

Curlew sandpiper from Sweden belonging to the EAF showed only close to significant three-year periodicity ($p = 0.08$) and a highly significant nine-year periodicity. Red-necked stint from NWA showed a non-significant tendency for 3 year periodicity as well as curlew sandpiper and sanderling from South Africa (Appendix S2).

Applying a 20- years moving window on the juvenile percentage data for curlew sandpiper from Sweden revealed that only starting years in the late-1950s until early-1970s yielded a significant periodicity of three years. Also when applying wavelet analysis on the juvenile percentage data for curlew sandpiper from Sweden along the EAF, we again found a strong periodicity, changing from five years in the late-1950s to three years by the mid-1960s, followed by a gradual fading out after the late-1970s (Fig. 2).

In the EAAF, a 20-years moving window of autocorrelation in the red-necked stint dataset from SEA, showed a gradual weakening of the three-year periodicity, although also in early years, correlation was not significant. In the same species, wavelet analysis showed significant periodicity, changing from three to four, and again three years from the mid-1980s until approximately 2003. Curlew sandpiper and sharp-tailed sandpiper from SEA showed a significant periodicity for a very short time, ranging from two to four years between the late-1980s and early-1990s, however, when we divided the time series to 20-year windows, no significant autocorrelation emerged (Fig. 3).

Periodicity in Lemming abundance

Autocorrelation analyses on lemming abundance data for the breeding grounds resulted in a significant three-year periodicity for Taimyr Peninsula only, East Siberian Plains data showing a non-significant tendency for three year periodicity (Appendix S2). Wavelet analysis disclosed a significant three- year periodicity in lemming abundance on Taimyr Peninsula, the breeding area of curlew sandpipers from Sweden, until the early-1990s (Fig. 3).
2). Remarkably, this three-year periodicity was persistent also well after the periodicity in shorebird productivity had started to decline (figure 2).

**Figure 2:** The wavelet power spectrum, showing the change in periodicity for (A) curlew sandpiper from the East Atlantic Flyway and (B) lemming abundance index on Taimyr Peninsula. The likelihood of periodicity increases from blue to red and the black contour marks areas with >95% confidence. Grey broken line indicates the “cone of influence”. Outside this area inferences are weak due to edge effects. Juvenile percentages (A) and lemming index (B) are presented in black bars (Y-axes of the right). (C) Results of a 20-years moving window autocorrelation analysis for annual juvenile percentages of curlew sandpiper from the East Atlantic Flyway (EAF) and lemming abundance on Taimyr Peninsula, researching the presence of a three-year periodicity. Autocorrelation function for the three-year period in each 20-years window is plotted in the y-axis against the first year of the 20-years period in the x-axis (each data point represents the three year correlation coefficient of three-year periodicity in one 20-years window).

Although neither a significant autocorrelation was found in the complete time series of lemming abundance in Chukotka nor for the 20-years moving window analysis on the same dataset, wavelet analysis showed a 3-4 year cycle in lemming abundance for a short period from the mid-1970s until the late-1980s. In East Taimyr and the East Siberian Plains, no significant periodicity was found for the 20-years moving window analysis, and a very short period with approximate three year periodicity was detected around the late-1980s and late-1990s respectively for the wavelet analysis (figure 4). Also two short periods with four to six year periodicity were revealed in these areas, but these periods are outside the cone of
influence and may therefore be subject to an edge effect and should be interpreted with caution.

Most species in the EAAF do not correlate in their breeding success (Appendix S3) and we thus negate the possibility of one common, large-scale effect on the breeding success of these Arctic waders while on their breeding grounds. Summer temperatures had a significant positive effect on breeding success in five out of nine cases in our study species, with June and July having the strongest effects (Table 1). In two cases (ruddy turnstone from SEA and curlew sandpiper from NWA) some ORs for monthly temperature averages on the breeding grounds were <1 indicating a negative effect of temperature on breeding success. However, the overall summer temperature effect was always positive (i.e. the net OR obtained by multiplication of both ORs was >1). The breeding success of bar-tailed godwit from NWA was not affected by temperatures whereas the population of the same species from SEA was
affected by July temperatures. These two populations breed in widely separated Arctic areas, East Siberia and Alaska respectively, thus explaining these differences. Remarkably, the same applied to red-necked stints, where only the population from NWA was affected by May temperatures, although their breeding grounds are assumed to overlap. Conversely, for curlew sandpipers from SEA and NWA, which are also thought to belong to the same population, June temperatures had the most significant effect on birds from both wintering locations. Predation index did not have a significant effect on any species from the EAAF. In the EAF, using the 45 years of data available for curlew sandpiper from Sweden, we found a significant negative effect (i.e. OR<1) with 50% reduction in breeding success due to predation pressure on Taimyr Peninsula (Table 1).

**Figure 4:** The wavelet power spectrum, showing the change in periodicity in lemming abundance index in three areas on the breeding grounds of waders from the East Asian-Australasian Flyway (EAAF). The likelihood of periodicity increases from blue to red and the black contour marks areas with >95% confidence. Grey broken line indicates the “cone of influence”. Outside this area inferences are weak because of edge effects.
Discussion

In contrast to the suggestions made by the *Alternative Prey Hypothesis* and to the previous findings along the EAF (Blomqvist et al. 2002), we could not identify a strong, common link between breeding success in Australian wintering waders and predation pressures on their Arctic breeding grounds. Given presumed periodicity in lemming numbers and corresponding periodicity in predation pressure (Roselaar 1979, Summers 1986), periodicity in the breeding success of Arctic breeding birds is considered to be one of the hallmarks of the major impacts that lemming cycles have on Arctic ecosystems (Ims and Fuglei 2005). Our (re-) analysis of breeding success in waders along both the EAAF and the EAF now highlights that these cycles have changed in some populations in the last 30 years, alternating between periodic and aperiodic years, and may explain the lack of correlation between breeding success across the various wader species and populations in the EAAF (Appendix S3) and with lemming-linked predation indices from the breeding grounds (Table 1). Clearly, the interaction between lemmings-predators and waders is more complex than a simple predator-prey relationship. Waders are assumed to have a weaker link as they may be a secondary alternative prey in the presence of goose colonies (McKinnon et al. 2013). However, in the general absence of high density goose colonies in the eastern Russian Arctic, where our study species mainly breed, waders are most likely to be among the preferred alternative prey.

For the EAF, only the Swedish data set for curlew sandpipers is sufficiently long to allow a robust analysis of the possible change in periodicity in breeding success over time. The remarkable change in periodicity in this long term data set (Fig. 2), combined with (i) the tendency for a three-years periodicity in two species from South Africa during 1969-1986 (Appendix S2), and (ii) the decreasing trends in periodicity in red-necked stint and the waning periodicity in curlew sandpiper and sharp-tailed sandpiper from SEA in the EAAF (Fig. 3A-B), infer a loss of periodicity in breeding success of Arctic breeding waders in both flyways.

A problem with our analysis is that it is difficult, if not a statistical impossibility, to prove the absence (rather than the significant existence) of a pattern. The use of a qualitative, expert-opinion based index of lemming abundance may at least in part have contributed to weakened patterns. Yet, this does not explain why these patterns vary over time and space. Although our analysis suggests similarities in the effect of changing lemming cycles on waders of the EAF and EAAF, there may be inherent differences between their typical breeding areas that
may anyhow result in a weaker periodicity for waders in the EAAF compared to the EAF. Lemmings are a characteristic feature within the lowlands of the central and northern belts of the Arctic tundra. Typically, their peak occurrences are least pronounced in the southern regions of their distribution and in valleys or coastal sections separated by mountains. It therefore seems that rather systematic periodicity of lemmings is an attribute of large uniform land masses like Taimyr Peninsula (P. Tomkovich, pers. observ). Such is also evident from our analysis, where only lemming abundances in Taimyr Peninsula showed a persistent three-year periodicity.

Waders from the EAAF use more extensive breeding areas than species from the EAF. Finding correlations between breeding success of an entire population and predation index thus requires substantial spatial synchronization in lemming cycles and predator abundances. Although synchronization of lemming cycles has been shown for some areas in the Arctic (Angerbjorn et al. 2004), a phenomenon that is probably driven by predators’ mobility and weather conditions across large spatial scales (Krebs et al. 2002), the likelihood of such synchronization is probably diminishing with an increase in area. Still, there we observed changes in periodicity over time and we should not rule out that these could be due to change in climatic conditions in some areas, possibly altering patterns of synchrony (Krebs 2013), leading to weaker cycles on large spatial scales.

Despite the relatively short time series, notably for species from NWA, limiting the power of our analysis, our results demonstrate a positive effect of Arctic summer temperatures on breeding success for some waders in the EAAF as shown previously for waders in the EAF and the EAAF (Boyd et al. 2006, Tulp 2007). The three different spring/summer months included in our analysis represent different phases in the breeding season of Arctic waders. Temperatures in late May determine the timing of snowmelt and the rate of thawing. Prolonged snow cover affects the availability of nesting locations and food, and may delay breeding attempts (e.g. Niehaus and Ydenberg 2006). June and July are the peak egg laying and chick rearing periods respectively, during which high temperatures may positively affect breeding success (e.g. Krijgsveld et al. 2003, McKinnon et al. 2012).

Changes in lemming cycles have a cascading effect on linked species in the Arctic (e.g. resident grouse species (Kausrud et al. 2008). Recently, changing lemming cycles have been linked to decreasing populations of brent geese Branta bernicla bernicla L. wintering in Europe (Nolet et al. 2013). These changes were also suggested to affect red knot migrating
through the United States (Fraser et al. 2013). However, there is little evidence of recent changes in lemming cycles across the Canadian Arctic and other studies point at changes occurring on the stopover areas of red knot in North America (e.g. Baker et al. 2004), in combination with Arctic snow conditions (McGowan et al. 2011) as the leading factor for population decline.

Here, we enhanced the analysis of former studies and suggest an effect of fading lemming cycles on Arctic breeding birds of the East Atlantic and the East Asian-Australasian Flyways. We argue that fading out of lemming cycles in some parts of the Arctic is responsible for faltering periodicity in wader breeding success along both flyways, and is a major factor for changing breeding conditions of Arctic nesting birds. These changed conditions have not yet resulted in any marked changes in trends on breeding success across years and declining numbers of waders along the EAAF are therefore more likely related to changing conditions at stop-over and wintering sites (e.g. Fernandez and Lank 2008, Norris 2005, Rogers et al. 2010) or yet undetected changes in breeding success on the Arctic breeding grounds.

Acknowledgements

We thank the many volunteers from the Victorian Wader Study Group and from the Australasian Wader Studies Group for collecting the juvenile percentages data in Australia and the large number of contributors to the Arctic Breeding Conditions Survey for the lemming abundance data. We thank Peter Biro and Jan Van-Gils for useful comments on previous drafts of this manuscript and Simeon Lisovski for his help in preparation of the figures. The study by MS and PT was supported by RFBR, research project No. 12-04-01526 A.
Table 1: Odd Ratios for the best quasi-binomial logistic models relating juvenile ratios in wintering wader populations with predation index (P) and average monthly temperatures in May ($T_{May}$), June ($T_{June}$) and July ($T_{July}$) within the population’s respective breeding region. Numbers in brackets are 95% confidence intervals. N is the total number of observations on which juvenile ratios were based across all years (No. of years) used in the model for which data was available for all four predictors. Data are presented for six species along the East Asian-Australasian Flyway (EAAF) wintering in south east Australia (SEA), three species along the EAAF wintering in north Western Australia (NWA), and one species along the East Atlantic Flyway (EAF) observed in Sweden. Hyphens indicate that the parameter was not selected into the final, best model.

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Appendices

Appendix S1: Lemming abundance indices for east Siberia (from central Taimyr to Bering Strait) and Alaska.

Table A1: Indices indicate lemming abundance as (1) low, (2) average and (3) high; although intermediate classifications are possible due to data averaging within regions. Numbers in superscript indicate sources.

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\(^1\) Pozdnyakov (2004); \(^2\) Chernyavsky & Lazutkin (1999); \(^3\) Koroleva (2002); \(^4\) Chernyavsky & Lazutkin (2004); \(^5\) Volpert & Shardina (2002); \(^6\) Kuryshnev & Dorogoi (1989); \(^7\) Dorogoi, (1988); \(^8\) Fischer, Stehn & Walter (2010); \(^9\) Blomqvist et al (2002); \(^10\) Arctic Birds Breeding Conditions Survey (ABBCS); \(^11\) Vladimir Pozdnyakov (pers.comm).
Appendix S2

Figure A1: Autocorrelation function (ACF, the correlation between a variable and itself in two different time points) and partial autocorrelation function (PACF, the correlation between a variable and itself in two different time points, once correlation between all successive lags are controlled) for annual juvenile percentages of waders from the East Asian-Australasian Flyway (EAAF). ACF (left panels) and PACF (right panels) between juvenile percentages for species from South East Australia (SEA) (species names depicted in panels) are plotted against time lag (years; a time lag of 0 years yielding a correlation coefficient of 1). Dashed lines indicate the confidence limit, values above these lines are significant (P<0.05%).
Figure A2: Autocorrelation function (ACF, the correlation between a variable and itself in two different time points) and partial autocorrelation function (PACF, the correlation between a variable and itself in two different time points once correlation between all successive lags are controlled) for annual juvenile percentages of waders from the East Asian-Australasian Flyway (EAAF). ACF (left panels) and PACF (right panels) between juvenile percentages for species from north Western Australia (NWA) (species names depicted in panels) are plotted against time lag (years; a time lag of 0 years yielding a correlation coefficient of 1). Dashed lines indicate the confidence limit, values above these lines are significant (P<0.05%).
Figure A3: Autocorrelation function (ACF, the correlation between a variable and itself in two different time points) and partial autocorrelation function (PACF, the correlation between a variable and itself in two different time points once correlation between all successive lags are controlled) for annual juvenile percentages of waders from the East Atlantic Flyway (EAF). ACF (left panels) and PACF (right panels) between juvenile percentages for species from Sweden and South Africa (SA) (species names depicted in panels) are plotted against time lag (years; a time lag of 0 years yielding a correlation coefficient of 1). Dashed lines indicate the confidence limit, values above these lines are significant (P<0.05%).
**Figure A4:** Autocorrelation function (ACF, the correlation between a variable and itself in two different time points) and partial autocorrelation function (PACF, the correlation between a variable and itself in two different time points once correlation between all successive lags are controlled) for lemming abundance index in six breeding areas in the Alaskan-Siberian Arctic. ACF (left panels) and PACF (right panels) between lemming abundance for areas depicted in panels are plotted against time lag (years; a time lag of 0 years yielding a correlation coefficient of 1). Dashed lines indicate the confidence limit, values above these lines are significant (P<0.05%).
**Appendix S3**: Correlation matrix for juvenile percentages of the East Asian-Australasian Flyway

**Figure A5**: Spearman rank correlation matrix for juvenile percentages of species from south east Australia (SEA). Significant correlation after Bonferroni correction for multiple comparisons was found only between ruddy turnstone and curlew sandpiper ($r=0.78$, $p<0.001$).
**Figure A6:** Spearman rank correlation matrix for juvenile percentages of species from north Western Australia (NWA). None of the results were significant after Bonferroni correction for multiple comparisons.
Chapter 3

A latitudinal trend in migratory fuel deposition rate as driver of trans-equatorial long distance migration in shorebirds

Yaara Aharon-Rotman, Ken Gosbell, Clive Minton and Marcel Klaassen

2 Author contributions: MK and YAR originally formulated the idea. CM and KG conducted field work for retrieval of geolocators with help from YAR and MK. YAR performed statistical analysis with advice from MK. YAR wrote the manuscript with advices from all other authors.
Abstract

Trans-equatorial long-distance migrations of high-latitude breeding animals have been attributed to narrow ecological niche widths. We investigated an alternative hypothesis suggesting that trans-equatorial migrations result from the possibility that the rate at which body stores to fuel migration are deposited increases with absolute latitude; we thus suggest that longer, trans-equatorial migrations may actually enhance fuelling rates and therewith the chances of a successful, speedy and timely migration back to the breeding grounds. To this end we first explored the earlier-suggested existence of a latitudinal trend in fuel deposition rate in a global dataset of free-living migratory shorebirds. We next tested two predictions on how such a trend should impact the migratory itineraries on northward migration under the time-minimisation hypothesis, using 56 tracks of high-latitude breeding shorebirds migrating along the East Asian-Australasian Flyway. We found a strong positive effect of latitude on fuel deposition rate, which probably relates to latitudinal variations in primary productivity and available daily foraging time. We next confirmed the resulting predictions that (1) when flying from a stopover site towards the equator migrants use long jumps that will take them to an equivalent or higher latitude at the opposite hemisphere, and (2) once having passed the equator, the migrant will use small steps, basically fuelling only enough to make it to the next suitable staging site. These findings may explain why migrants migrate “the extra mile” across the equator during the non-breeding season in search of better fuelling conditions, ultimately providing secure and fast return migrations to the breeding grounds in the opposite hemisphere.
Introduction

Migration has evolved as an adaptation towards avoiding risks, such as unfavourable thermal conditions, food shortage, predation and disease, as well as seizing opportunities where and when they arise (e.g. Salewski and Bruderer 2007, Louchart 2008). For many high latitude breeders, notably cold and food shortage are important drivers of movements towards the equator before the winter sets in (Newton 2003). Even if wintering at high latitude is still an option, the advantages in thermostatic cost close to the tropics may outweigh the extra costs of flight (Wiersma and Piersma 1994). However, these explanations for the seasonal long-distance migration from high latitudes fail to explain long-distance trans-equatorial migrations as found in many animal taxa. The prime reason suggested for these extra-long, trans-equatorial migrations is that migrants have narrow ecological niche widths, using equivalent habitats (to which they are specialised) at both sides of the equator during the most productive season (Newton 2003). But in some cases, such as in many Arctic-breeding, long-distance migratory shorebirds, apparently suitable and similar habitats are passed, or temporarily used and then left, for more southern destinations. Possibly counter-intuitively, we here build a case that these long trans-equatorial flights actually enhance the chances of a successful, rapid and timely migration back to the breeding grounds.

The main migratory habits of shorebirds have likely evolved with time constraints being the major selective force (Alerstam and Lindström 1990, Lindström and Alerstam 1992). Notably shorebirds breeding at high latitude are thought to be particularly hard-pressed for time during spring migration. A time-minimization strategy (Hedenstrom and Alerstam 1997) is essential to enable a timely arrival at the breeding grounds to provide optimal use of the short seasonal peak in food abundance, which is of vital importance to the birds’ breeding success. Therefore, poor quality feeding sites should be used less intensively and possibly even be skipped during spring migration, depending on the relative quality across sites along the flyway and the distances between them.

A typical feature of migratory birds, as well as some other migrant taxa, is the build-up of fuel stores in preparation for migration. Fuel deposition rate or “fuelling rate” is dependent on body mass and importantly determines speed of migration (Lindström 2003) and migration strategies. These strategies can be divided according to energy requirements and flight distances into three categories; hop, skip and jump, from the less energetically demanding strategy to hop short distances, to the energetically challenging strategy of accumulating large
stores of extra fuel to jump long distances between sites (Piersma 1987). Whether a migrant adopts a hop, skip or jump strategy is importantly determined by the difference in quality of sites along the flyway. Under the time-minimisation hypothesis, the two extremes, hopping and jumping, will evolve if the potential for fuelling is evenly or unequally distributed along a flyway, respectively. The energetic costs associated with carrying extra fuel from particularly good staging sites is rewarded with a faster migration compared to when a hopper strategy had been used. The maximum extent of such jumps is ultimately limited by physiological and flight mechanical constraints on fuel accumulation.

Although fuelling rates can be modified by variations in daily energy expenditure, metabolised energy intake is considered the most important determinant of fuelling rates (Lindström 2003). Thus, it is assumed that variations in fuelling rates between sites are geared to local differences in food availability (e.g. Schaub and Jenni 2001, Piersma et al. 2005, Van Gils et al. 2005). Fuelling rates also show significant correlation with absolute latitude in red knot Calidris canutus worldwide (Piersma et al. 2005) and western sandpiper Calidris mauri in America (Williams et al. 2007). On the other hand, Schaub and Jenni (2001) and Lyons et al. (2008) did not find a significant correlation between fuelling rates and latitude in semipalmated sandpipers Calidris pusilla in North America and four species of passerine birds migrating between Europe and Africa, respectively. We suggest that latitudinal patterns in fuelling rates, if substantiated, could have considerable repercussions for the migratory strategy of these birds and notably for the distances covered in each migratory leg and overall migration speed.

We predict that for an optimal, time-minimising migrant facing latitudinal variation in potential fuelling rates: (1) when flying from a stopover site towards the equator during northward migration towards the breeding grounds, the migrant will use big jumps that will take it to an equivalent or higher latitude in the opposite hemisphere, and (2) once having passed the equator, the migrant will use small steps, fuelling sufficiently only to make it to the next suitable staging site (to avoid the energetic cost of carrying excessive amounts of fuel). If these predictions are true, it may be beneficial to spend the non-breeding season away from the equator at higher latitudes in search of better fuelling conditions. Although thus covering a longer distance, this longer journey may result in a higher net speed of migration during the return migration to the breeding grounds.
The aim of this study is to comprehensively investigate (a) the effect of latitude on fuelling rates in different migratory shorebirds at a global scale, (b) investigate the potential underlying factors relating to such a pattern with respect to latitude, i.e. (i) total intertidal biomass in different sites worldwide as an estimation of prey availability, and (ii) primary productivity (measured using chlorophyll-a concentration) and (c) test whether a latitudinal pattern in fuelling rates lead to the above predicted (1-2) migration strategies using satellite transmitter data and geolocation dataloggers (‘geolocator’) data for three high-latitude breeding shorebird species migrating along the East Asian-Australasian Flyway.

Methods

Fuel deposition rate (“fuelling rate”) data (expressed as mass increase in grams per day, g d⁻¹) and lean body mass data for migratory shorebirds was collected from the literature for different geographical locations (Zwarts et al. 1990, Gudmundsson et al. 1991, Piersma et al. 2005, Ens et al. 2006, Lindström et al. 2011, Ma et al. 2013) (Fig. 1). The data was based on mass gain within staging shorebird population or from average mass gains in re-trapped individuals.

Published data on total intertidal biomass of benthic macrofauna (g ash free dry mass [ g AFDM] m⁻²) (“total biomass”) worldwide was collated (Warwick and Ruswahyuni 1987, Piersma et al. 1993, Masero et al. 1999, Ricciardi and Bourget 1999, Kober and Bairlein 2006, Jing et al. 2007, Purwoko and Wolff 2008) (Fig. 1). Only data collected during the periods in which actively migrating shorebirds might have been present was used.
Figure 2: Sampling locations of total intertidal biomass (g AFDM m^{-2}; dark grey circles) and sites where fuelling rates were measured (mass gain in g d^{-1}; light grey circles). All data was obtained from the literature (see text for details).

Primary productivity in the areas where data on total biomass and fuelling rates were collected (limited to 25 km from the coast line) was downloaded from Ocean Productivity (http://www.science.oregonstate.edu/ocean.productivity/index.php). These primary productivity data were remotely estimated as a function of chlorophyll-a, available light, and the photosynthetic efficiency averaged over 8 days. These descriptions are based on the Vertically Generalised Production Model (VGPM) (Behrenfeld and Falkowski 1997).

To investigate whether latitudinal variation in potential fuelling rates has the predicted effects on migratory strategies, as outlined in the introduction, the sequence of sites used on northward migration for three shorebird species was analysed. Only stop-over sites that were visited for a duration of at least 4 days were considered because by stopping for less than 4 days, the bird is likely to gain only very little mass (Warnock 2010). The location of each stopover site was used to calculate the distance covered in each leg (i.e. distance between each stopover site). Routes were reconstructed from data downloaded from geolocators deployed and retrieved by the Victorian Wader Study Group in South Australia (for ruddy turnstone *Arenaria interpres* and sanderling *Calidris alba*) and in Victoria and Tasmania (ruddy turnstone). Of the 56 tracks available for ruddy turnstone five have been published.
previously and details on these tracks can be found in Minton et al. (2010). Although the increasingly shrinking weight and size of geolocators currently allows them to be fitted on bird species as small as 15g, their accuracy is limited to about 200km of latitude and 50km of longitude (Welch and Eveson 1999, Phillips et al. 2004). In our study, birds on northward migration are travelling over a total distance exceeding 13000 km. During this journey they stop and refuel at about 3-4 sites along the route. The spatial accuracy of geolocators is therefore sufficient for our specific aim of analysing the distance travelled between each stop-over site.

Although much higher spatial accuracy can be attained with satellite transmitters, their weight prohibits its use in the majority of wader species. We used published routes based on satellite transmitters of two subspecies big enough for this tracking technique, the bar-tailed godwit species *Limosa lapponica baueri* and *Limosa lapponica menzbieri* (Battley et al. 2012).

**Statistics**

Because we expected a U-shape relationship with latitude (i.e. low values around the equator, increasing towards the poles), we used general linear modelling, second order polynomial models to examine the effect of latitude on fuelling rates, primary productivity and total biomass.

Body mass has been suggested to affect fuelling rates (Lindström 2003) and we therefore included lean body mass alongside latitude as predictor variables in our analyses of fuelling rates.

Also a phylogenetic mixed model was used to examine the effect of latitude and lean body mass on fuelling rates. This phylogenetic mixed model allows (random) phylogenetic effects to be included. Using R (R Core Team 2014), we fitted the polynomial model using a Bayesian approach by applying the MCMCglmm function within the MCMCglmm package. For the phylogeny, a tree based on data provided in Thomas et al. (2004) was applied (appendix S1). Parameter-expanded priors were specified following Hadfield (2010).

Prior to analyses, fuelling rates, lean body mass, primary productivity and total biomass were 10-log transformed to obtain normality in all models. Insignificant predictor variables were pruned from the models.
When closing in on their ultimate target destination (breeding grounds), the remaining distance to the ultimate target and thus also the potentially remaining maximum flight distance to that target, becomes progressively shorter. Under the assumption that birds will follow the shortest route to their ultimate target, when plotting leg flight distance against latitude it was expected that leg flight distances would fall on or below the line depicting the distance between the departure staging site and the ultimate target. For regressions between flight distance and latitude, $H_0$ for the slope is thus not the default zero, but rather half of the slope of the line depicting the maximum distance from any staging site to the ultimate target (Fig. 3).

All statistical analyses were conducted using R version 3.0.3 (R Core Team 2014)

**Results**

Using general linear modelling, lean body mass had a significant effect on fuelling rates ($\beta=0.56, p<0.01$). In the same model, latitude was significant only in its quadratic form ($\beta=0.0005, p<0.01$) (Fig 2a), suggesting a U-shaped relationship with the bottom located at the equator.

Accounting for phylogenetic effects using a phylogenetic mixed model, we found that again fuelling rates were positively correlated to latitude only in its quadratic form (slope: $\gamma = 0.0002; P_{\text{MCMC}} <0.001$). Contrastingly, however, body mass had no additional significant effect on fuel deposition rate.

**Figure 3**: The relationship between latitude and (a) fuel deposition rates (FDR), corrected for lean body mass (lbm), in 13 shorebird species measured as mass increase in g d$^{-1}$; (b) mean net primary productivity (NPP) at the sites where FDR and biomass data were collected, measured as a function of chlorophyll-a, available light, and the photosynthetic efficiency and (c) total intertidal biomass (biomass). All locations of data collection are shown in figure 1.
Latitude had a significant effect on primary productivity in the polynomial model (latitude: $\beta=0.00057$, $p<0.01$, latitude$^2$: $\beta=0.000033$, $p<0.01$), thus confirming a U-shaped relationship with latitude (Fig. 2b).

Latitude in its quadratic form had no significant effect on total biomass ($p=0.42$), whereas latitude had a significant effect ($\beta=0.025$, $p<0.01$). This implies that a latitudinal trend only exist in the northern hemisphere and not a U-shaped relationship as we had predicted (Fig. 2c).

After H$_0$ correction (see methods), the migration routes from geolocator and satellite transmitter data obtained from trans-equatorial migrants showed a significant negative relationship between flight distance and latitude during northward migration towards the breeding grounds (Fig. 3).
Figure 4: Flight distances (km) for each leg during northward migration. In each panel, the observed distance covered is plotted against the starting staging site latitude for the species: ruddy turnstone ($\beta = -76.3$, $F_{1,145}=337.1$, $p<0.01$), sanderling ($\beta = -34.6$, $F_{1,51}=29.6$, $p<0.01$), bar-tailed godwit from the sub species baueri ($\beta = -88.5$, $F_{1,23}=67.9$, $p<0.01$) and menzbieri ($\beta = -34.3$, $F_{1,20}=107$, $p<0.01$). In each panel the broken diagonal grey line represents the maximum distance from any staging site to the breeding grounds via a great circle route. Grey shaded areas represent low latitude regions (i.e. tropical, ranging between -20 and 20 degrees of latitude).
Discussion

In this study we showed that migration strategy in trans-equatorial migrating shorebirds is characterised by long migration legs (jumps) when leaving staging sites at temperate southern hemisphere latitudes, followed by shorter hops/skips towards the final destination, their Arctic breeding grounds. These shorter hops/skips after a major jump may be a result of fine-tuning timing of arrival at the breeding grounds. However, according to the time minimisation hypothesis, the long initial jump across the equator followed by shorter hops/skips may also result from a specific pattern in the potential for fuel deposition along the birds’ flyway. Using an extended data set, we confirmed earlier suggestions of a latitudinal trend in fuelling rate in shorebirds, where fuelling rates decrease from high to low latitudes in the northern hemisphere (Piersma et al. 2005, Williams et al. 2007, Lyons et al. 2008), but more importantly, we here show for the first time that this latitudinal trend exists in both hemispheres. Given this confirmed latitudinal trend in fuelling rates, the typical migration strategies presented here are consistent with the predictions of the time-minimisation hypothesis.

Although we could not confirm a correlation between fuelling rate and lean body mass using the phylogenetic model, the effect of body mass on fuelling rates has been suggested in earlier studies (e.g. Lindstrom 2003). Due to corrections for phylogeny, this effect may have been masked in our analysis, as closely related birds tend to be of similar size. The strong effect of body mass on fuelling rates using a polynomial model, excluding an effect of phylogeny, supports this inference.

There are a number of potential explanations for why latitude is such an important factor in determining fuelling rates. Firstly, although here confirmed only in the northern hemisphere, latitudinal trends have been shown in (surface) prey biomass (Piersma et al. 1993, Mathot et al. 2007, Purwoko and Wolff 2008) and now also in primary productivity in this study for both hemispheres. Secondly, since migrants tend to follow the wave of productivity shifting between the northern and southern hemispheres over the course of a year, they also tend to experience long days for most of the year. This is notably the case when they are staging at high latitudes during summer, which would consequently allow for increased feeding time (Bauchinger and Klaassen 2005). Thirdly, high foraging and feed-intake rates, the latter potentially resulting in high heat increments of feeding, might be constrained by high ambient temperatures (cf. heat dissipation limitation theory; Speakman and Król (2010); see also
Kurnath and Dearing (2013)). Indeed, Battley et al (2003) found intake rates in waders staging in tropical regions to be limited, for which heat stress was suggested to be an important factor. Low temperatures at high latitudes may assist in heat dissipation and thus promote higher fuel deposition rates. Finally, although explaining the increasing trend in fuel deposition rate in the northern hemisphere only, migrants may increase fuelling rate at higher latitude because they might become increasingly more pressed for time to reach the breeding grounds in time (Reneerkens et al. 2007, Williams et al. 2007).

Data on flight leg distance in relation to the latitude of the departure site (Fig. 3) confirmed our predicted effects of latitudinal variation in fuelling rates on migration strategies. These predictions were based on combining the time-minimisation hypothesis and the positive relationship between fuelling rate and absolute latitude. Most individual shorebirds start their northward migration from their relatively high latitude non-breeding site, towards the breeding grounds with a jump strategy. This jump is followed by a skipping/hopping strategy towards the breeding ground after passing the equator. Not all individuals follow the shortest distance from their wintering grounds to the breeding grounds. This is evident from the points above the line depicting the distance between the departure staging site and the ultimate target for the two subspecies of bar-tailed godwit (Fig.3). These detours from the “shortest-distance-to-goal” may actually result in a higher net speed of migration due to better feeding conditions (Alerstam 2001).

Many migrants forage more or less continuously while migrating, with food being regularly distributed along their migratory path, reducing the immediate need for the build-up of migratory stores. Still, also in these cases of continuous food availability but with variations in the amounts of food in space, the time-minimisation hypothesis would expect the skipping or jumping of areas with relatively low food availability. For terrestrial migrants, migration range is importantly restricted by ecological barriers, such as oceans and deserts. But given the latitudinal trends in productivity, it might be expected that similar patterns are present in trans-equatorial migration strategies in other bird groups and marine animal taxa. Indeed, this is also the case in Short-tailed Shearwaters (*Ardenna tenuirostris*) which travel south, presumably to exploit highly productive Antarctic water to gain sufficient weight to migrate through the less productive waters of the tropics (Carey et al. 2014). Generally, mass-specific energetic costs of locomotion decrease with animal size, being lowest in swimmers, followed by flyers and finally animals using terrestrial locomotion (Schmidt-Nielsen 1972, Hein et al. 2012). From an energetic cost perspective marine species might therefore also engage in
trans-equatorial migrations driven by latitudinal patterns in food availability. However, many marine species have anti-tropical distribution patterns (i.e. the same species have separate populations on both sides of the equator). The lower speed of locomotion in swimmers relative to flyers may restrict the distance covered within the annual cycle (Alexander-McNeill 2002, Johansson et al. 2014).

The latitudinal pattern in the suitability of staging sites for migratory fuelling confirmed here may have been of great importance in the evolution of migratory strategies. This is highlighted by the migration itineraries of some shorebird species en route from their southern hemisphere non-breeding areas to their Arctic breeding grounds. Therefore, relatively high latitude staging sites (farther from the tropics) may also be of particular importance for the successful completion of migratory journeys. Migratory shorebirds along the East-Asian Australasian Flyway, which includes the species analysed in this work, are facing many challenges. The largest disturbances and threats are observed in the staging sites in the Yellow Sea area, located at about 38°N. These sites may therewith currently be acting as the primary ecological bottlenecks for the migratory shorebirds that have relied on them in the past, also explaining their downward population trends (R. Fuller pers. comm; MacKinnon et al. 2012). The here proposed concept of migratory birds disproportionately relying on high latitude staging areas thus needs to be considered when prioritising conservation efforts.

Acknowledgement

We are grateful to Dr. Anthony Ricciardi for sharing his world-wide data on macrobenthic biomasses. We thank the Victorian Wader Study Group and all the volunteers for their huge efforts in the field in putting and retrieving geolocators, Simeon Lisovski for his help in downloading primary productivity data, Nick Clark and Vincent Careau for their help in running the phylogenetic mixed models, and Graeme Hays for his comments on a previous version of the manuscript.
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Appendix

Appendix S1: Phylogenetic tree used for the phylogenetic mixed model, pruned from Thomas, Wills & Szekely (2004).
Chapter 4

A chain is as strong as its weakest link – assessing the consequences of habitat loss in long-distance migratory shorebirds

Yaara Aharon-Rotman, Silke Bauer and Marcel Klaassen

³Author contributions: MK and YAR originally formulated the idea. SB developed the Stochastic dynamic model. YAR run all model scenarios with input from MK and SB. YAR wrote the manuscript with advices from all other authors.
Abstract

Migratory species are threatened worldwide. The conservation of these species represents a major challenge, as they use multiple sites, all contributing to varying degrees in sustaining high survival and reproductive success in an interdependent manner. There is particular concern for shorebirds of the East Asian-Australasian Flyway (EAAF), where numbers of migratory species have declined rapidly over the past few decades, the decline being mostly attributed to habitat loss along the East-Asian coast. Using a Stochastic Dynamic Programming -migration model, we assessed the consequences of a range of habitat degradation scenarios along the EAAF on migration behaviour, survival and reproductive success of long-distance migrating shorebirds. We used ruddy turnstone Arenaria interpres as our model species, since extensive tracking data of this species along the EAAF has become available over recent years, allowing model validation. Using the default parameter settings in our model, predictions largely matched empirical observations. Following manipulation of habitat quality through changes in intake rate, we found that decreasing intake rates on the wintering ground in South Australia, the only wintering ground in our model, had the highest negative effect on reproductive success and survival. We also identified Taiwan and the Yellow Sea as sites with high importance for reproductive success. Although habitats along the East-Asian coastline are currently most threatened from a range of global change processes, our modelling study also highlights the importance of conserving high-quality shorebird wintering habitat in Australia. This may be of notable importance to trans-equatorial shorebird migrants, which often make a long non-stop flight from their wintering grounds in order to skip low-latitude sites that typically provide little food.


**Introduction**

Many of the world’s migration systems are threatened (Wilcove and Wikelski 2008). Their reliance on a chain of suitable habitats along their migratory flyway rather than a single habitat where they reside during their entire lifetime, makes migratory species more vulnerable to habitat loss than non-migratory species (e.g. Cresswell 2014, Runge et al. 2014). Although one may argue that relying on one site is more risky, the probability of a site to be affected by environmental changes increases with the number of sites used (“multiple jeopardy”) (Newton 2004), putting long-distance migrants that use multiple habitats, at greater risk (Cresswell 2014).

On the other hand, the capacity to move away from deteriorating environments and the multiple possibilities to evade an unaffected site if a single site has deteriorated may be beneficial for migratory animals in the face of global changes (Runge et al. 2014). Indeed, some migrants have been shown to adjust their behaviour in response to changing habitat conditions in some situations, e.g. by changing distribution on the non-breeding grounds to use better feeding opportunities (e.g. Austin and Rehfisch 2005, Maclean et al. 2008, Verkuil et al. 2012). Theoretical models, however, indicate that unless changes are at an appropriate time-scale with the birds’ ability to evolve, abrupt changes may be too rapid to cope with (Klaassen et al. 2006).

Intermittent sites where migrating animals stop to rest, drink and feed (so-called ‘stopover’ or ‘staging’ sites), are essential for replenishing body stores in preparation for the next migratory leg. If migrants are not able to satisfy their energetic requirements, this can have direct and immediate consequences for survival but also knock-on to affect survival and reproduction at a later stage (“carry over effects”) (Gill et al. 2001, Warnock 2010). For long distance migrants, stopover sites where environmental conditions are deteriorating may thus constitute ecological bottlenecks, potentially having population level consequences. Recent global changes are likely to have an adverse effect on migratory species through habitat loss and degradation, changes to phenology and disease prevalence, impeding the migrants’ survival and reproductive success (Klaassen et al. 2012).

A timely arrival at the breeding grounds is advantageous for many migrants, notably those breeding at higher latitudes (e.g. Tulp 2007, Newton 2008), low migration speed resulting
from low fuelling rates (Lindström 2003) may be one of the causes for failing to reproduce successfully.

Many migratory shorebirds (also referred to as ‘waders’) are threatened (IWSG 2003, Sanderson et al. 2006). There is particular concern for the East Asian-Australasian Flyway (EAAF), where numbers of migratory species declined rapidly over the past few decades (MacKinnon et al. 2012). Habitat loss along this flyway has been suggested as the prime reason behind these declines (Barter 2002, Barter et al. 2003, van de Kam et al. 2010). The majority of this recent habitat loss has occurred in China, where a seawall along 60% of its 18000 km coast line was built to support the rapidly increasing urbanization and economy (Ma et al. 2014) resulted in a loss of 51% of all coastal wetlands over the past half century (MacKinnon et al. 2012), notably in the Yellow Sea, where 28% of tidal flats existing in the 1980s had disappeared by the late 2000s (Murray et al. 2014). Future reclamation projects in the area and elsewhere along the flyway (e.g. Qinghuangdao harbor master plan and Tianjin coastal leisure tourism area master plan) pose even more threats to migrants (See review on current and future plans for land reclamation of intertidal habitats along the flyway in MacKinnon et al. (2012)).

Conservation plans for migratory animals require several specific aspects to be considered. Firstly, migratory systems may be complex, consisting of many potential staging sites (for shorebirds along the EAAF ~700 suitable sites are known (Bamford et al. 2008)) allowing for a myriad of potential migration itineraries. Each migration itinerary may have different fitness consequences through the possibility of carry over effects, thus also having important consequences for population dynamics (Norris 2005). Secondly, sites may differ in their importance for migrants – there may be key sites whose loss would be detrimental while changes at other sites have hardly effects at all. Obviously, effective conservation requires the identification of such key sites and measures should be targeted particularly at them.

Traditional, single-site based approaches may thus not be the tools of choice and we therefore require methodology that considers (1) the whole flyway (2) the dependencies between sites in their use by migrants and (3) the role of multiple, simultaneously occurring threats. More advanced methodologies are needed, leading to informed conservation prioritisation and actions targeted at key stopover sites that make part of the best migratory itineraries i.e. that form the strongest chains (Runge et al. 2014). In this the conservation plan should manage
the impact of the threats in the light of their ultimate fitness consequences, i.e. in terms of survival and reproductive success (Webster et al. 2002, Cox 2010).

Stochastic Dynamic Programming (SDP) can adequately be used to capture a migrant’s behaviour, allowing the mapping of animal movement during a migratory season as a function of the characteristics of the migrant and its environment (Weber et al. 1998, Klaassen et al. 2006, Bauer et al. 2010). The basic components of SDPs are (i) state variables, which may describe the potentially changing physiology and morphology of the animal and its environmental characteristics, (ii) decision making based on the animal’s state and (iii) on costs and benefits of the actions in terms of a currency, which relates to the birds fitness (e.g. expected reproductive success). In other words, at any point in time the animal makes decisions based on its current state and internal and external constraints, ultimately leading to the maximization of the currency. Using SDP we can thus investigate the impact of changing conditions at stopover sites on the migration behaviour of migrants and also predict their survival and reproductive success as a function of these changes (Bauer and Klaassen 2013).

Using a SDP-migration model, we here aim at assessing the consequences of habitat loss and degradation along the EAAF on migration behaviour, survival and reproductive success of an exemplary long-distance migrant shorebird. We used the ruddy turnstone *Arenaria interpres* as our model species as extensive tracking data have become available for birds over recent years but generalize our results to other migrants with comparable ecological characteristics.

**Model system and methods**

**Model description**

The general framework of the SDP optimal migration model has been described earlier (Weber et al. 1998). We here therefore only briefly outline the model’s fundamental characteristics, describing the model and parameter estimations in detail in Appendix S1. The model covers the spring migration from the wintering (major non-breeding) grounds in east South Australia to the high-latitude, northern hemisphere breeding grounds. We refer to the Australian non-breeding grounds as “wintering” areas throughout this paper, as these sites are used during the northern hemispheric winter.

We used South Australia (SA) as the only wintering ground for ruddy turnstone. As a breeding site we used the average location in the centre of the breeding area as described by
Lappo et al. (2012). Potential stopover sites were identified using light-sensing geolocators. Between the years 2009-2014, The Victorian Wader Study Group have equipped 230 geolocators on ruddy turnstones and later retrieved 85 (37%). Complete individual annual routes were obtained from 56 individuals from South Australia (17), Flinders (Victoria; 4), King Island (Tasmania; 34) and Killarney beach (Victoria; 1). The use of geolocators ensured that the chosen sites are used by birds from the EAAF that made a full annual trip. To minimize the number of potential stopover sites in the model, sites which were used by low number of individuals or sites that were visited for <4 days only, were excluded from the model. The birds did not use exactly the same sites along the flyway. To further limit the number of sites within the modelling framework while accounting for as many individuals as possible, we clustered the positions of the sites visited by the geolocator birds within areas with a maximum radius of 500km to represent one stopover site. To account for all potential stopover sites, we also considered sites of international importance where high numbers of ruddy turnstone have been recorded (Bamford et al. 2008). This procedure resulted in seven stopover sites: north Western Australia (NWA), Indonesia, the Pacific Islands, Taiwan, Tokyo Bay, north Yellow Sea (NYS), Sakhalin and Alaska (Fig.1). The centre of these areas was used as the geographic position of the site and used to calculate migratory distances between sites (Fig. A1). All sites were characterized by site-specific food availability, energy expenditure and predation risk (see “parameterisation”).

We set the starting date of spring migration to the 4th of March and allowed it to last for a maximum of 100 days. The starting date was chosen because fuelling in preparation for migration cannot coincide with the energetically expensive process of moult, which ends in early to mid-February after a duration of about 120 days for most shorebirds (Summers et al. 1989, Minton et al. 2006b). For each day during this migration period, until their arrival on the breeding grounds, a bird was characterized by its body stores and location (site).

Within each time step, i.e. one day, a bird decided either to remain and forage on its present site, or to continue migration to another site. The decisions depended on the bird’s current state, i.e. body stores, time step and expected conditions on the present and subsequent sites. Once the bird reached the breeding grounds, we no longer modelled its decisions, but assumed that its reproductive success was determined by body stores and arrival time.
Body stores accumulation, energy expenditure and intake rates were all converted to a single unit and expressed in body stores per day (grams of fuel per day, which represented 32.77 kJ/g based on Klaassen et al. (1990)).

**Figure 1:** The East Asian-Australasian Flyway with potential stop-over sites for Ruddy Turnstone used in the model. Potential stop-over sites are based on both results of geolocator as well as sites of internationally importance where high numbers of Ruddy Turnstone have been recorded (Bamford et al. 2008). Site names depicted in numbers: (1) South Australia (2) north Western Australia (3) Indonesia (4) Pacific Island (5) Taiwan (6) Tokyo (7) north Yellow Sea (8) Sakhalin (9) Alaska (10) Breeding ground.
Parameterization

We estimated site-specific food availability following the latitudinal trend in fuelling rates found in chapter 3. In short, fuel deposition rate in shorebirds was found to correlate with absolute latitude, increasing from the tropics to the poles. We used this model to predict fuelling rates in the potential wintering and stopover sites along the flyway in the dynamic migration model (see Appendix S2 for details).

We estimated daily energy expenditure (DEE) and its temporal dynamics following Wiersma and Piersma (1994) and Piersma (2002). Monthly mean temperature, solar radiation and wind speed at each site were downloaded from (http://climexp.knmi.nl/). DEE is largely invariable in the tropics and changes with the course of the season in the northern and southern hemispheres, i.e. with a minimum during summer and a maximum during winter. Intake rates were then calculated as the sum of potential fuelling rates (g/day) and daily energy expenditure (Appendix S2).

Predation risk was assumed to increase with the abundance of predators (background predation risk), foraging intensity, and body condition (Witter and Cuthill 1993). All three aspects were used in assessing total predation risk (see Appendix S1 for details). Unfortunately, empirical data on variations in predation risk along the EAAF do not exist, and we therefore estimated the coefficients for background predation risk, foraging intensity-dependent and mass-dependent predation risk such that survival in the model approximately fit empirical estimates of survival during spring migration (Appendix S1).

We used empirical data on migration routes and timing of migrations of ruddy turnstone as derived from geolocators retrieved by the Victorian Wader Study Group in South Australia, Victoria and Tasmania to calibrate our model predictions using the standard parameter settings (“baseline model”). Departure date, length of stay at each stopover site and the sequence of sites visited were extracted from these data.

Model scenarios

To evaluate the consequences of varying environmental conditions, we increased and decreased intake rates in each site separately by 10% and 20%. We also reduced intake rates in each site until the site was no longer visited.
In a second step, we ran a series of scenarios where intake rates were reduced at two sites simultaneously. To this end we again step-wisely reduced intake rates by 10% and 20% in multiple sites along the route, for all 21 possible pairs of sites.

Finally, to imitate a complete habitat loss of sites, we set intake rates to zero for both single sites and two sites simultaneously.

For each scenario, we analysed migratory behaviour (i.e. sequence of sites visited and length of stay in each site), survival rate and reproductive success. Survival was the proportion of the population arriving at the breeding grounds alive. Reproductive success was estimated as a function of body condition and arrival time at the breeding grounds (Appendix S1).

Results

Empirical data showed that the majority of birds departed the wintering grounds in SA around the 20th of April and made a non-stop flight to Taiwan, where they staged for 10.8 days on average (including birds that did not visit the site). Indonesia was visited by 10% of all birds and the average overall staging time for all individuals (including birds which did not visit the site) was only 2.7 days. The next major stopover site was located in the north Yellow Sea with 68% of birds staging for >4 days with an overall average staging duration of 10.1 days. Sakhalin was used by 25% of birds staging for >4 days, with overall average staging duration of 1.5 days (Fig.2). Using the default parameter settings in the model (“baseline model”, Appendix S1,S2), predictions largely matched empirical observations. We found that ruddy turnstones were expected to depart from their wintering site in SA around the 15th of April after which they flew via Taiwan (where they staged for seven days on average), north Yellow Sea (11 days) and Sakhalin (22 days) to the breeding grounds. A mismatch between model predictions and empirical data was the staging duration in Sakhalin, where model predictions were considerably higher than shown by the empirical data (Fig.2). Average survival for the baseline model was 98% and reproductive success was 99%.

Scenarios involving increased intake rates at any site resulted in unchanged, high reproductive success (97-100%); survival was also unchanged and remained high (96-100%; Fig.3). In SA, departure date was 10.8 and 3.8 days earlier when intake rates increased by 10% and 20%, respectively. When intake rates increased by only 10% in NWA, i.e. a site not used in the baseline model, birds stayed there for over 30 days. A similar pattern appeared for Indonesia, where an increase in intake rates of 10% resulted in a 19 days use of the site,
which was not used in the baseline model. We also found that following an increase in intake rate by 10% and 20%, the length of stay in north Yellow Sea increased by 7.8 and 10.6 days, respectively (Fig.2).

Figure 2: A-I Staging durations following a change in food intake rates for the six staging sites used during northward migration. In each panel, staging duration (days) in the site mentioned in the title of each panel are plotted against the change in food intake rates (%. Zero represents no change from the baseline model). The colour of the line depicts the site in which the change in food intake rate took place. For example, in panel A, reducing intake rate in South Australia by 20% leads to no migration. In the same panel, increased intake rate in N.Western Australia (NWA) by 10% leads to reduction in staging duration in South Australia to slightly over 10 days on average. The same reduction also leads to increased staging duration of 30 days on average in NWA (panel B). The empirical average for staging duration, analysed from birds equipped with geolocators, is plotted in grey line (+- SD, in grey box) in each panel. In sites names, SA= South Australia and NYS= north Yellow Sea.
Reducing the quality of the wintering grounds in SA had a major impact on reproductive success and survival. A reduction of intake rates in SA by only 10% already resulted in early departure from SA and the use of NWA for an average of about 40 days, whereas NWA was not used in the baseline model (Fig.2). More importantly, reproductive success decreased by as much as 30% in this scenario (Fig.3). When intake rates in SA were further reduced by a total of 20%, the birds were not able to fuel up fast enough to start migration and the scenario resulted in 100% mortality.

Reduced intake rates in each of the stopover sites had a smaller effect compared to reduced intake rates on the wintering grounds in terms of survival and reproductive success. In most cases, birds skipped a stopover site used in the baseline model if its intake rates were reduced by 20%. Taiwan was an exception, as birds still visited the site following a 20% reduction in intake rates and only skipped this site when intake rates were reduced by 30% or more (Fig.2). However, despite continued use of the site, decreasing intake rates in Taiwan by 20% did result in increased use of NWA (which was not used in the baseline model). A decrease in the quality of Taiwan by 20% also resulted in 20% decrease in reproductive success (Fig.3). A 20% reduction in intake rates in Sakhalin lead to the use of Alaska, which was not used in any other scenario, as an alternative staging site for Sakhalin.

Figure 3: (A) Overall mortality and (B) reproductive success (%) are plotted against changes in intake rate in each site mention in the legend (%; Zero represents no change from the baseline model).
When intake rates were reduced at two sites simultaneously, any combination that involved reductions of intake rates on the wintering grounds (SA) by 10% resulted in reductions in reproductive success by 19-41% (Fig.4). Any combination of 10% reduction of intake rates that involved Sakhalin or Taiwan (but not SA) also resulted in reduced reproductive success by up to 17%. When we reduced intake rates further down to 20%, the effect on reproductive success was not markedly stronger and resulted in 26% reduction (Fig.4). Effects on survival rates were negligible in the 10% reduced intake rate scenarios and only occurred in the combination of SA and NWA (27%). In the 20% reduction in intake rates, a part from the expected 0% survival in any combination that involved SA, there was no effect on survival in any other combination (Fig. A2, Appendix S3).

![Figure 4: Matrix of reproductive success (%) in the model, following a change in intake rate in two sites (mention in the axis) at the same time. In the upper part from the diagonal, results for changing intake rate in -20% for two sites at a time are depicted. Lower part of the matrix (below diagonal) represents reproductive success in the model after reducing intake rates by 10%. Diagonal represent reproductive success in the baseline model (i.e. original intake rate in each site), thus reproductive success is constant over the diagonal (99%). Pacific Island is not shown in the figure as the birds never used the site. All combinations involved 20% reduction in intake rates in South Australia resulted in reproductive success =0 (red colour). All combinations involved 10% reduction in intake rates in South Australia (except Yellow sea), resulted in lower reproductive success (yellowish colour). Also combinations of 20% reduction in intake rates in Sakhalin together with Taiwan, Yellow sea and Alaska resulted in reduced reproductive success (74%, 77% and 75%, respectively). All other combinations resulted in reproductive success> 80%.

To simulate complete habitat loss, stopover sites were excluded from the model in pairwise combinations. If not excluded, Taiwan and Sakhalin were always used in all combinations. Although empirical data show that Indonesia is used by some individuals, it was never used in any of the scenarios. We also found that excluding Taiwan always resulted in the use of NWA (Fig.5). When both Taiwan and NWA were excluded, reproductive success was
reduced by 20%. Lowest reproductive success (75%) was found when north Yellow Sea and Sakhalin were excluded simultaneously. All combinations in which Taiwan was excluded, also resulted in low reproductive success (78-86%) (Fig. 5). Effect on survival was found for the combination of excluding Alaska and Sakhalin (14%). Any other combination did not result in any change in survival rate from the baseline model.

**Figure 5:** An overall description of consequences of sites exclusion, in combinations of two sites at a time. Each line represents a scenario, in which the sites (x-axis) symbolized with x are excluded. On the right y-axis, reproductive success for this scenario is represented. The size of the point in each site is in accordance with the length of stay. Top two scenarios are empirical data, based on geolocators results (grey circles), and baseline model, using original intake rates (red circles).


Discussion

The most distinctive outcome of our modelling study is the outstanding importance of high-quality wintering grounds for breeding success and survival of ruddy turnstones. We found that a reduction of intake rates on the wintering grounds lead to a disproportionate reduction in reproductive success. Conditions in wintering habitats have previously been suggested to importantly affect migrant populations (Cresswell 2014), and have been shown to affect survival and arrival date to the breeding ground in, e.g. American redstarts *Setophaga rutilicilla* (Marra et al. 1998, Norris et al. 2004), Black-tailed Godwits *Limosa limosa* (Gill et al. 2001, Alves et al. 2013) and Redshank *Tringa tetanus* (Burton et al. 2006). These carry over effects operate on individual body condition with individuals enjoying favourable wintering conditions attaining higher body condition for migration and breeding (Smith and Moore 2003, Alves et al. 2013). Our results also demonstrate strong effects of habitat deterioration further down the flyway, at distinct stop-over sites. For instance, also decreased habitat quality in Taiwan and Sakhalin strongly affected reproductive success. This highlights the importance of maintaining favourable conditions at crucial staging sites in addition to good wintering conditions, for safeguarding a high breeding success and survival. This is not merely a theoretical outcome of a modelling exercise, but exemplified in a number of migratory systems (Baker et al. 2004, Amano et al. 2010). For instance, decreased intake rates due to overharvesting of horseshoe crabs in Delaware Bay, an important stopover site for Red Knot *Calidris canutus* in the US, caused a reduction in survival by up to 37% as well as a reduction in reproductive success for the years 2000-2002 (Baker et al. 2004). In the EAAF, Amano et al. (2010) suggested that changes/deteriorations along the Yellow Sea as well as on mudflats in Japan may predict a decline in population of some shorebird species.

Overall, our baseline model predictions were consistent with empirical findings using geolocators. The only major deviation was the staging duration in Sakhalin, which were considerably longer in the model than shown in empirical data. This might be explained by the fact that the model ends when birds arrive at the breeding grounds and thus, does not consider any additional foraging after arrival on a breeding or pre-breeding site. Thus, in the model the last possibility to fuel up and acquire a good body condition before breeding is on Sakhalin. Klaassen et al. (2001) found that Arctic waders are ‘income breeders’ and produce eggs from local resources, which they acquire at or close to the breeding grounds. It is therefore likely that this model assumption may not always hold true, the breeding grounds
potentially providing food to locally top-up and build on their body stores to improve body condition immediately preceding breeding.

Although migratory species differ in their ecologies, e.g. diet, energy costs of flight, or site fidelity, the outstanding importance of high-quality wintering grounds for survival, successful migration and high reproductive success that we found in the model probably applies to other shorebird species. This may be of notable importance to trans-equatorial shorebird migrants, which often make a long non-stop flight from their wintering grounds in order to skip sites providing little food at low latitudes. However, it should be born in mind that our predictions of food availability along the flyway are based on general correlations between intake rates across latitudes. These are overall trends, and in reality, there may be local deviations at specific sites, e.g. when key sites provide particularly good staging conditions exceeding those predicted by the general relations. For example, fuelling rates for red-knot *Calidris canutus rufa* in Delaware Bay, an important stopover site for red-knot in America, were estimated to be 4.6 g d\(^{-1}\) (Piersma et al. 2005), double than the rate we would predict according to the general latitudinal trend (2.27g d\(^{-1}\)). Red- knot *C. c. piersmai* and *C. c. rogersi* from the EAAF are dependent on a relatively small area in Bohai Bay in the Yellow Sea, supporting 45% of the flyway population during northward migration. Red- knots are amongst the latest to start their northward migration from their wintering grounds, thus having little time for staging and fuelling while en route, requiring sites allowing for high fuelling rates to migrate successfully (Battley et al 2005). It is therefore assumed that the Bohai Bay mudflats allow birds to achieve high fuelling rates by providing exceptionally high quality food for red-knot, which are highly specialised and forage mainly on shellfish (Rogers et al. 2010, Yang et al 2013). For Great Knot *Calidris tenuirostris* for example, fuelling rates were estimated at 3.55g d\(^{-1}\) in Yalu Estuarine Wetland in north Yellow Sea (Ma et al 2013). Clearly, loss of such highly preferred sites may result in a significant population decline.

The SDP modelling framework allows for adaptive flexibility of individual migrants in response to changes. It inherently assumes birds to be omniscient of conditions along their flyway, migrating as to maximise life-time reproductive success. Thus, if there are changes at one or more sites along the flyway, it is also assumed the birds are aware of the “new” conditions and respond optimally to it. For instance, if conditions on an intermediate site were assumed to deteriorate as in our scenarios, birds in the model side-stepped this site and used a site with ‘unchanged’ better conditions instead, sustaining high survival and
reproductive success. This assumption of adaptive responses may be violated, particularly when faced with novel or sudden environmental changes. Using a similar SDP framework, Klaassen et al. (2006) found that following organized scaring by farmers in Norway, naïve pink-footed geese had a marked response and much lower expected fitness than experienced geese that were able to anticipate and adapt to changes. Whether, and to which degree, real migrants can anticipate and respond to environmental changes remains difficult to assess.

Many shorebirds are site faithful to their stopover and wintering sites (e.g. Warnock and Takekawa 1996, Leyrer et al. 2006, Minton et al. 2006a, Buchanan et al. 2011), which indicate limited ability of seeking alternatives as a response to deteriorating conditions. Site-faithfulness may persist irrespective of better options being available and hamper their survival probability if a displacement is forced due to habitat loss (Burton et al. 2006). In contrast, some migratory waders have been shown to shift their non-breeding distribution in response to changing environmental and climatic conditions (e.g. Austin and Rehfisch 2005, Maclean et al. 2008, Verkuil et al. 2012), indicating some degree of flexibility in response to environmental conditions. In other groups of migratory birds, such as in many migratory passerines, adaptive changes in the phenology of departure from the non-breeding grounds and arrival at the breeding grounds have been shown in response to changing environmental conditions (e.g. Tøttrup et al. 2008). However, whether these adaptive changes are sufficient or not to fully compensate for the changes in the environmental conditions remains to be shown (Both and Visser 2001).

Conserving migratory species represent a major challenge, as they use multiple sites in an interdependent manner and which may contribute to varying degrees to sustaining high survival and reproduction. Conservation actions involving migratory animals must therefore explicitly account for the animal’s’ movement in space and time and take the dependencies among sites into account (Runge et al. 2014). An example of an action reminiscent of this is to limit wind turbines during peak migration of birds and bats (Drewitt and Langston 2006). In this paper we have also shown that changes in feeding opportunities on the wintering grounds may have detrimental impacts on populations of long-distance migratory shorebirds. The potential impact of proposed projects that may affect migratory species on the wintering grounds, such as the plan to harvest seaweed on the beaches of South Australia (http://www.judayang.com/en/index.asp), should be meticulously scrutinized, carefully considering the timing, movement and requirements of migratory species in the areas where seaweed harvesting is planned. Future work should focus on researching and gathering data
on carrying capacity (e.g. food availability and fuelling rates) in sites along the flyway. Together with the advancement in tracking devices technology for small birds, this will allow applying our method to different species and assist in prioritizing conservation efforts.
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Appendices

Appendix S1 – Model description

We used dynamic programming (Clark & Mangel 2000; Houston & McNamara 1999) to find the sequence of migratory decisions maximising the fitness of shorebirds under the environmental conditions encountered during spring migration. The model follows earlier versions (Bauer et al. 2010; Bauer et al. 2008; Weber et al. 1998) but has been slightly modified for its application on ruddy turnstone.

We distinguished distinct sites including the wintering grounds, staging sites and the breeding grounds (see Fig. A1 for site locations and Table A1 for distances between sites). The migration period was divided into whole days \( t \) covering the period from 4 March to 12 June, i.e. 100 days.

We assumed that at time \( t \), the expected future fitness \( F \) of a female shorebird, is a function of its fuel stores \( x \), and its location \( i \): \( F(x,t,i) \). Body stores \( x \) may vary between 0 g, where the bird used in our simulations has a total body mass of 100 g and dies of starvation, and \( x_{\text{max}} \), where it reaches the maximum fuel load of 100 g or 3277 kJ, equivalent to a total body mass of 200 g. Thus, the energetic equivalent of 1 g body stores (and, thus, 1 \( x \) in the model) is 32.77 kJ. All energetic variables in the model, e.g. intake rate or energy expenditure, were expressed in terms of \( x \) (and thus \( g_{\text{fuel}} \)) over time, i.e. \( g_{\text{fuel}} \) d\(^{-1}\).

At the breeding or destination site \( N \), the expected future fitness or terminal reward is \( F(x,t,N) \). For each time step when the bird has not yet arrived at its destination, it has two behavioural options: either foraging at intensity \( u \) (0 \( \leq \) \( u \) \( \leq \) 1) or, if fuel stores permit, flying to another site.
**Terminal Reward**

If a bird has reached the breeding site \( N \), we no longer model its decisions but assume that part of its current reproductive success will be determined by arrival time \( K(t) \), and its body reserves at arrival, \( K(x) \). For \( K(x) \) we used:

\[
K(x) = \begin{cases} 
\frac{1}{2} (e^{w(x-x_c)} - e^{-w(x-x_c)}) + 1 & \text{if } x \geq 10 \\
0 & \text{if } x < 10
\end{cases}
\]  

(eqns. A1)

Waders are income and not capital breeders (Klaassen et al. 2001). The scaling parameters \( w \) and \( x_c \) were set to 0.028 and 10, respectively, which ensures that also birds arriving with body stores as low as \( x=10 \) would still have a potential chance of reproductive success (i.e. \( K(x) = 0.5 \)), gradually increasing with body stores to \( K=1 \) for animals arriving with maximum fuel stores (i.e. \( x = 100 \)).

In addition to body stores reproductive success was also conditional on the timing of arrival. Breeding success in waders is highly correlated with early arrival (Tulp 2007). However, arriving too early is disadvantageous due to snow cover and low food availability. We therefore allowed for successful breeding only if birds arrive at the breeding grounds within a narrow time window:

\[
K(t) = \begin{cases} 
0 & \text{if } t < t_{\text{min}} \text{ or } t > t_{\text{max}} \\
1 & \text{if } t_{\text{min}} \leq t \leq t_{\text{max}}
\end{cases}
\]  

(eqns. A2)

The optimal period of arrival at the breeding grounds was therefore set between 31\(^{st}\) May and 9\(^{th}\) June (\( t_{\text{min}}=89, t_{\text{max}}=98 \)). We assumed that arriving at the breeding grounds outside this period led to breeding failure in the present year. Birds that failed to reach the breeding grounds in time or arrived with body stores lower than \( x=10 \) had an expected fitness of \( B_0 = 3 \) - they may not reproduce this year but might still do so in future years.
Thus ultimately the terminal reward was defined as:

\[ F(x, t, N) = K(x) \times K(t) + B_0 \]  

(eqn. A3)

**Foraging**

The maximum intake or gain rate that a foraging shorebird may attain is site- and time-dependent \( g(u, i, t), \text{ g}_\text{fuel} \text{ day}^{-1} \) and are provided in Fig A1. We assumed stochastic differences in maximum intake rate and the SD of \( g \) for all sites and times arbitrarily to 1 \( \text{ g}_\text{fuel} \text{ day}^{-1} \). Accordingly, we modelled the intake rate as a discrete random variable with outcomes \( g_1(i), \ldots g_j(i), \ldots, g_{\text{max}}(i) \) and the probability of achieving a particular maximum intake rate is given by:

\[ P[G = g_j(i)] = p_j(i), \text{ where } \sum_j p_j(i) = 1 \]  

(eqn A4)

The actual intake rate is determined by foraging intensity \( u \). How much of this intake rate ultimately is stored as body stores depends on the energy expenditure \( e(i, t), \text{ (g}_\text{fuel} \text{ day}^{-1}) \), which mainly depends on ambient temperature (see S2 for site-specific estimates of intake rate and energy expenditure). Consequently, if a bird with body reserves \( x \) forages with intensity \( u \) at site \( i \), its body reserves in the next time-step will be:

\[ x + u g_j(i) - e \]  

(eqn. A5)

Maintaining fuel stores and foraging at certain intensity incurs a fitness cost in terms of increased risk of predation and injury (Witter & Cuthill 1993), which may vary between different locations. Thus, the total predation risk depends on a site-specific baseline predation risk, \( b_0(i) \), the foraging intensity \( (u) \) dependent risk, \( b_1(i) \cdot u^m \), and the mass (i.e. fuel-store \( x \)) dependent predation risk, \( b_2(i) \cdot x^n \), combined in summative manner since foraging or carrying high fuel loads can independently increase predation risk.
\[ m(x, u, i) = b_0 + b_1 \frac{(x + u^g(i, t) - e)^{a_1} - x^{a_1}}{(a_1 + 1)(u^g(i, t) - e)} - b_2 u^{a_2} \]  

(eqn. A6)

For ruddy turnstones we used the following settings in eqn A6: \( a_1 \) and \( a_2 = 2 \); for all sites we used the same site-specific coefficient for background predation risk \( b_0 = 1.0^{-4} \); for foraging-intensity dependent predation risk \( b_1 = 1.0^{-5} \); for mass-dependent predation risk \( b_2 = 1.0^{-4} \). These settings ensure that adult survival over the period considered in the model would always be >90%.

The optimally behaving bird should forage with an intensity \( u \) that yields the maximum expected fitness:

\[ H_f(x, t, i) = \max_u [(1 - m(x, u, i)) \cdot F(x + u^g(i, t) - e(t, i, t + 1, i))] \]  

(eqn. A7)

Flying

Alternative to staying and foraging an individual could depart to the next site depending on its fuel stores \( x \) and the distance \( D (\text{km}) \) to the next site. Its fuel stores upon arrival at the destination \( x_a \) were calculated using:

\[ x_a = \left( \frac{c^2}{(c - (c(1 - (1 + x / x_{\text{max}})^{-0.5} - D))^2 - 1)} \right) \cdot x_{\text{max}} \]  

(eqn. A8)

where \( c \) is the flight range parameter which was calculated using

\[ c = \frac{D_{\text{max}}}{1 - (x_f / x_{\text{max}})^{0.5}} \]  

(eqn. A9)

where \( D_{\text{max}} \) was the maximum flight range when dedicating fraction \( x_f \) of the maximum fuel load \( x_{\text{max}} \) to flight. For the ruddy turnstones in this study we used \( x_f = x_{\text{max}} \) and \( D_{\text{max}} = 8000 \), resulting in \( c = 27314 \) km. These settings were based on empirical data from geolocators,
where individual ruddy turnstones were recorded flying non-stop from South Australia to Taiwan, a distance of about 7600km (Minton et al. 2010). These parameters also matched flight costs estimated for waders engaging in long-distance migration by Hedenström (2010).

If an individual decided to depart, it should fly to the site $j$ yielding the maximum expected fitness at the destination:

$$H_d(x, t, i) = \max_j [F(x_a, t + \sum_{z=1}^{j-1} D_z / v), j]$$  \hspace{1cm} (eqn. A10)

where $v$ is flight speed, which was estimated at 1440 km/d (Piersma & van de Sant 1992).

The optimal decision is the behavioural alternative, foraging or departing, yielding the highest future expected reproductive success:

$$F(x, t, i) = \max \{ H_f(x, t, i), H_d(x, t, i) \}$$ \hspace{1cm} (eqn. A11)

The optimal decision matrix for all combinations of time, site and body stores was used in subsequent forward simulations and thus, allowed us to track individual birds during their spring migration. Consequently, we predicted staging times and body store dynamics from these simulations and compared these with empirical data. In the forward simulation we followed individual birds on their migration to the breeding grounds using a Monte-Carlo simulation method. To this end, we let a population of 100 individuals with initial body stores start in a wintering site at $t=0$. Thereafter, using the optimal decision matrix, all individuals performed the optimal action according to their present body stores, site and time, i.e. migrating to site $j$ or foraging with intensity $u$. The fuel gain at a given site was determined
Appendix S2: Site-specific energy budgets: intake rates and energy expenditure

*Intake rates*

We estimated maximum site-specific fuel deposition rate ($FDR_{\text{max}}$; expressed as mass gained in g d$^{-1}$) based on the correlation established between fuel deposition rate (FDR, g d$^{-1}$) in free-living shorebirds and the absolute latitude ($L$, ° N/S) at which those measurements took place (Chapter 3). This correlation showed that FDR is proportional to $e^{0.027 \times L}$. Lindström (2003) estimated the relationship between $FDR_{\text{max}}$ (g d$^{-1}$) and lean body mass (BM, g) for individual shorebirds as 0.23 BM$^{0.66}$, without taking a potential effect of latitude into account. For a ruddy turnstone with a lean body mass of 100 g this equates to an $FDR_{\text{max}}$ of 4.7 g d$^{-1}$. To acquire latitude specific $FDR_{\text{max}}$ for the ruddy turnstones in our model from our latitude specific FDR estimates, we arbitrarily fixed Lindström’s estimate to hold for birds at the Arctic circle (i.e. at 66.6 °N), so that:

$$FDR_{\text{max}} = 0.787 e^{0.027 \times L}$$

(eq. A12)

To obtain estimates of intake rates (Fig. A1), we added $FDR_{\text{max}}$ to the estimates of daily energy expenditure at the specific period, $t$, in time.

*Energy expenditure*

Daily energy expenditure (DEE) is determined by maintenance metabolism (MMR) and the energy required for certain activities such that typically $\text{DEE} = 1.7 \times \text{MMR}$ (Piersma 2002). Wiersma and Piersma (1994) and Piersma (2002) estimated DEE using a red-knot mount in a range of microhabitats and constructed a model allowing the calculation of DEE from air temperature, wind speed and solar radiation. To calculate DEE for Ruddy Turnstones we
followed the calculation procedures in Wiersma and Piersma (1994). The conductance at a
given wind speed and solar radiation depend on microhabitat and we thus calculated these
coefficients as the average of multiple habitats that suit ruddy turnstone: mudflats and bare
salt marsh, on snow on tundra, behind tundra hillock, on tundra hillock, dense group, open
group, solitary, head in wind, flank in wind (average coefficients of 0.007541 and 0.000907
for wind speed and solar radiation, respectively). Body temperature was set to 41° after
Wiersma and Piersma (1994) for red knot. Monthly mean temperature, solar radiation and
wind speed at each site were downloaded from (http://climexp.knmi.nl/). According to the
advancement of seasons, DEE is largely invariable in the tropics and changes with the course
of the season in the northern and southern hemispheres, i.e. with a minimum during summer
and a maximum during winter (Fig. A1). Thus, the net fuelling at a given site and time of the
year results from the difference between the site-specific intake rate and energy expenditure -
both depending on local conditions, e.g. temperature.
Figures

**Figure. A1.** Default values for daily intake rates ($g_{\text{fuel day}^{-1}}$) and daily energy expenditure (DEE, $g_{\text{fuel day}^{-1}}$) for all wintering and migratory staging sites as a function of date. Intake rates were estimated from predicted fuelling rates based on the correlation between fuelling rates and absolute latitude in shorebirds found in chapter 3. DEE was estimated from site- and date-specific ambient temperatures, solar radiation and wind speed. Sites names are: NWA= north Western Australia, Pacific= Pacific Islands, NYS= north Yellow Sea.
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Appendix S3: Mortality rate following a change in intake rate in combination of two sites at a time

Figure A2: Matrix of mortality rate in the model, follow a change in intake rate in two sites (mention in the figure) at the same time. In the upper part from the diagonal, results for reducing intake rate by 20% for two sites at a time. Lower part of the matrix (below diagonal) represent mortality rate in the model after reducing intake rates by 10%. Diagonal represent mortality rate in the baseline model (i.e. original intake rate in each site), thus mortality rate is constant over the diagonal (99%). Pacific Island is not shown in the figure as the birds never used the site.

All combinations involved 20% reduction in intake rates in South Australia resulted in 100% mortality (grey colour). All combinations involved 10% reduction in intake rates in South Australia resulted in higher mortality, especially in the combination with north Western Australia (NWA; 27% mortality). Other combinations did not lead to marked change in mortality rate than the base line model.
References


Chapter 5

Why fly the extra mile? Using stress biomarkers to assess wintering habitat quality in a migratory shorebird, the Ruddy Turnstone, *Arenaria interpres*

Yaara Aharon-Rotman, Katherine L. Buchanan, Nick Clark, Marcel Klaassen and Willian A. Buttemer

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**Author contributions**: MK and YAR originally formulated the idea. YAR conducted field work. WAB, KLB and YAR performed the lab work of stress biomarkers. NC performed all parasite screening. MK and YAR developed the fuelling rate estimations. YAR performed statistical analysis. YAR wrote the manuscript with advices from all other authors.
Abstract

Migratory birds make decisions about how far to travel based on cost-benefit trade-offs. However, in many cases the net effect of these trade-offs is unclear. We sought to address this by measuring feather corticosterone (CORT), leucocyte profile, parasite infection levels and estimating fuelling rates in three populations of ruddy turnstone Arenaria interpres, which are migratory shorebirds. These birds fly from the high-Arctic breeding ground to Australia, but differ in that some decide to end their migration early (Broome, north Western Australia), whereas others travel much further to either South Australia or Tasmania. We hypothesized that the extra costs in birds migrating greater distances and overwintering in colder climates would be offset by benefits when reaching their destination. This would be evidenced by lower stress biomarkers in populations that travel further, owing to the expected benefits of greater resources and improved vitality. We show that physiological stress measurements were lower in birds flying to South Australia and Tasmania than those overwintering in Broome. Furthermore, our modeling predicts that birds in the more southern locations enjoy higher fuelling rates. Finally, lower parasitic infection prevalence, although not statistically tested, also support the benefits of flying further south. Our data are consistent with the interpretation that birds occupying more costly wintering locations in terms of higher migratory flight and thermoregulatory costs are compensated by better feeding conditions and lower blood parasite infections, which facilitates timely and speedy migration back to the breeding ground.
Introduction

Many long-distance avian migrants exploit seasonally favourable conditions at high northern latitudes for breeding. On completion of breeding, declining food abundance and the onset of lower temperatures drive them to migrate southward (Newton 2003), where the reduction in thermoregulatory costs close to the tropics may outweigh the costs of flying (Wiersma and Piersma 1994). Species vary in their extent of southward migration, with some species and populations of the same species remaining at north-temperate locations, while others may fly to the tropics, or further still, cross the equator to over-winter in the higher latitudes of the southern hemisphere (Newton 2003).

Large-scale variation in conditions across sites results in different energy costs and benefits across the migratory range (Quaintenne et al. 2011). Carry-over effects (i.e. events that occur in one season but affect individual success in following seasons) may play an important role in population dynamics (Norris 2005), with individuals selecting pre-migratory staging areas at better sites being more likely to attain higher body condition prior to departure and thus better survival and breeding success (Marra et al. 1998, Smith and Moore 2003, Alves et al. 2013). Given the substantial differences in the flight costs involved in reaching these different wintering sites (major non-breeding sites) and the associated latitudinal differences in temperature, there are likely to be offsetting benefits for Arctic-breeding birds that choose south-temperate latitudes for their wintering destinations. Such benefits might include improvements in food quality and quantity, a reduction in the extent of infection and disease, and lower predator abundance.

In Chapter 3 we found a significant relation between latitude and fuel deposition rates in shorebirds, which may explain the longer flights taken by trans-equatorial migrants. We showed that, despite the extra flight distance, the benefit of wintering in high-quality sites may actually enhance the chances of a successful, rapid and appropriately timed migration back to the breeding grounds. The other hypothesised factors mentioned above that would make it worthwhile to fly the extra mile still remain to be investigated.

The ruddy turnstone *Arenaria interpres*, a trans-equatorial migrant and an Arctic circumpolar breeder, is an ideal species to examine the potential benefits for individuals that elect to migrate greater distances to the south-temperate locations. This species has accessible
populations that winter in the Australian tropics, as well as others that fly 2800 and 3300 km further southward to coastal locations in South Australia and Tasmania, respectively.

In this study we indirectly evaluated the vitality of populations in Broome, South Australia (SA), and King Island, Tasmania (KI) by quantifying selected stress biomarkers. These included assessments of haematological parameters such as relative white blood cell (leukocyte) concentration (WBC) and level of haemosporidian blood parasite infection of the genera *Haemoproteus* and *Plasmodium*.

Relative WBCs from blood smears provide quantitative information on circulating immune cells (Campbell 1995), which are strongly affected by glucocorticoid levels. Therefore, they are widely used in vertebrates as a measure of long-term stress (Gross and Siegel 1983, Maxwell 1993, Dhabhar et al. 1995), particularly in birds (reviewed by Davis et al. 2008). Following a stress event, there is typically an increase in the ratio of heterophils to lymphocytes (H/L ratio) due to changes in cell trafficking and redistribution between the blood and other immune compartments (Dhabhar et al. 1995, Dhabhar 2002). H/L ratios have been used to assess susceptibility to disease and infection (e.g. Davis et al. 2004, Al-Murrani et al. 2006) and to indicate immune challenge (Krams et al. 2012). These ratios are also known to be affected by growth and reproduction (e.g. Moreno et al. 2002, Lobato et al. 2005). The use of eosinophil counts as an indicator of stress is not as common, although studies have demonstrated that stress-induced glucocorticoid secretion leads to a reduction in eosinophil numbers in mammals (Gordon 1955) and amphibians (Belden and Kiesecker 2005). The function of basophils is still only poorly understood. It is probably involved in acute inflammatory defence and immediate hypersensitivity reaction (Maxwell & Robertson 1995, Vinkler et al. 2010). Moreover, leukocyte profiles may also change as a response to disease. Therefore, quantifying the number of eosinophils and monocytes may help distinguish the effects of disease from those of stress (Jain 1986).

Unlike the glucocorticoid response to stress, which starts within 2-5 minutes of a stress event (Romero and Reed 2005), changes in the leukocyte population in the peripheral blood are not evident until 1-2 hours afterwards (e.g. Dhabhar et al. 1995, Cirule et al. 2012) and remain altered longer than plasma glucocorticoid levels (McFarlane et al. 1989). However, the timing of these responses varies among taxa (Romero and Reed 2005, Davis et al. 2008).

Following the arrival on their wintering grounds, long-distance migrants may be particularly susceptible to parasitic infections, in part due to their immune system dealing primarily with
muscle injuries and fatigue suffered during extended flights (Piersma 1997). Therefore, the choice of wintering habitat may be important for minimising the rate at which these animals encounter harmful infections. This includes haemosporidian blood parasites of the genera *Haemoproteus* and *Plasmodium*, which are a diverse vector-borne group of parasites known to infect a wide range of avian taxa, including shorebirds (Clark et al. 2014). These parasites can adversely affect the fitness of their hosts (Valkiunas 2005), and potentially influence the migration patterns exhibited by migratory shorebirds (Piersma 1997, Figuerola and Green 2000). Although previous studies have found haemosporidian prevalence to vary among shorebirds having different habitat associations (Mendes et al. 2005b, Yohannes et al. 2009), few studies have directly tested for spatial differences in haemosporidian parasite load within a single species (but see D’Amico et al. 2008). Such information is necessary to determine whether differences in parasite pressure contribute to the different wintering strategies exhibited by shorebird populations.

In migratory birds, the glucocorticoid corticosterone (CORT) plays important roles in adjusting metabolism in preparation for long-distance migration and lipid use during the flight itself (Jenni-Eiermann et al. 2009). However, chronically elevated levels of CORT may have detrimental health consequences in regard to immune function, body condition, growth, reproduction and survival (Charmandari et al. 2005, Blas et al. 2007). Elevated levels of CORT are also observed in birds at sites with variable or unpredictable food supply, resulting in lower food intake rates (Reneerkens et al. 2002). Therefore, we also examined glucocorticoid content in feathers from birds at the two south-temperate sites, along with estimates of fuel deposition rates.

The use of feather CORT as a stress biomarker has gained prominence recently (Bortolotti et al. 2008, Bortolotti et al. 2009, Lattin et al. 2011) and has been used to assess survival in house sparrows (Koren et al. 2012), reproductive performance in tree swallows *Tachycineta bicolor* (Harms et al. 2010) and consequences of sex-specific parental care in nestling seabirds (Fairhurst et al. 2012). These steroid hormones are deposited into the feathers during feather replacement, therefore feather CORT should reflect the stress levels experienced by a bird over the moulting period (Sheriff et al. 2011). Ruddy turnstones moult their feathers at the wintering ground, not long after arrival. The duration of the moult period varies in response to a range of environmental factors (Remisiewicz 2011), but on average takes about 100 days (Dietz et al. 2014). Because turnstones moult exclusively at their wintering ground,
CORT concentration in feathers should reflect the stress levels these birds experience during the early months following their arrival to the wintering sites (September-December).

Because migratory birds must accumulate sufficient energy stores to fuel their flight requirements before departing a given staging area, fuel deposition rates (“fuelling rates”) importantly determine the speed of migration (Lindström 2003). Estimated fuelling rates at the three sites based on the relationship we found between fuelling rate and latitude in shorebirds (Chapter 3), suggest that conditions for building fuel stores in shorebirds wintering in Australia are best in Tasmania, becoming less favourable moving north.

The aim of this study is to assess the quality of three major wintering sites for ruddy turnstones: Broome, South Australia (SA) and King Island (KI) (Fig.1), using health measurements (stress biomarkers and blood parasite infection) and estimates of fuel deposition rates. We predict that following the latitudinal trend found in Chapter 3, overwinter habitats in higher latitudes will not only provide better feeding conditions for migrants, but also lower parasite infection rates. We therefore predicted that birds overwintering at such sites (SA and KI) will have lower stress biomarkers than birds at lower latitudes (Broome). If these hypotheses are proven correct, it will support the idea that migratory distance represents a trade-off between the costs of travel and thermoregulation relative to the benefits of greater resources and improved vitality.

**Methods**

**Sampling**

Birds were captured using cannon nets over the period of migratory preparation (March-April) between the years 2011-2013. Biometrics and blood samples were taken in Broome, north Western Australia 18°S 122°E, in Nene Valley, South Australia (SA) 38°S 140.5°E and King Island, Tasmania (KI) 40°S 143.5°E. We also collected four covert feathers (two from each side) from 66 individuals in South Australia and 194 individuals on King Island. Feathers were stored at room temperature until analysed.

All sampling was performed in accordance with the animal ethics committee at Deakin University (AEC project number B36-2012), and with permission from the Victorian Department of Sustainability and Environment #10006663, and Wildlife Ethics Committee of
South Australia (project number 35-2012).

(1) **Stress Biomarkers**

**Leukocyte concentration (circulating cellular immunity)**

We obtained differential leukocyte concentrations using blood smears from 23 individuals captured in Broome in 2012 as well as 40 birds from South Australia and 52 from King Island captured in 2013. Smears were air dried once made and were fixed in methanol for 30 seconds prior to staining. After staining (Giemsa Stain, Sigma-Aldrich) smears were examined at 1000x magnification with oil immersion and the number of heterophils, lymphocytes, eosinophils, monocytes and basophils were counted from the first 100 leukocytes encountered. All counts were made in a random order and were blind to the original sampling time point by YAR.

![Figure 1: The three wintering sites for ruddy turnstone in Australia mentioned in the text: Broome, north Western Australia (dark grey), South Australia (medium grey) and King Island, Tasmania (light grey).](image)

**CORT concentration in feathers**

**Assay protocol**

Feathers were prepared and assayed following Lattin et al (2011). Briefly, after removing the calamus, feathers were cut into small pieces <5mm² and placed in 25 ml flasks. We then added 7ml of methanol to each sample and placed the flasks in a sonicating bath for 30 minutes. The flasks were then placed on a vortex platform (Ratek Instruments, Boronia,
Australia) within an incubation cabinet that was maintained at 50°C overnight. Separation of
the methanol fraction was accomplished by vacuum filtration using a Buchner funnel fitted
with Whatman #4 filter paper. The extracted methanol was placed in a 100 x 13mm culture
tube and then dried under nitrogen gas on a block heater at 50°C. The dried extracts were
then reconstituted in Tris-HCl buffer and the CORT content determined using a standard
radioimmunoassay (RIA) using Sigma antibody (Sigma-Aldrich, Saint Louis, Missouri, USA,
product # C8784), following Lattin et al (2011). In order to quantify both intra-assay
variation and methanol extraction efficiencies in relation to feather mass, we created three
homogenous mixtures of feathers that were each pooled from carcases of three different
individuals. Feathers were cut in small pieces (<2mm) and were pulverized in a Ball Mill
(QIAGEN GmbH, Hilden, Germany). Samples from these pools were run in each assay to
determine inter-assay variation.

Samples were analysed in 4 assays and the mean 50% binding level was 2 ng/ml (range 1.5-
2.2). Samples testing the population differences were relatively balanced across two assays.
Extraction efficiency was determined by spiking each sample with 20μl of radiolabelled 3H-
CORT (2467.5 [±96.9] cpm) before extraction, and average recovery in reconstituted samples
was 82%. All final values were individually adjusted to account for actual recovery
percentages in each sample.

Validating an optimal feather mass for CORT evaluation

Lattin et al (2011) found that feather sample mass used in the extraction affected the
calculated concentration of CORT in Starling *Sturnus vulgaris* feathers. In order to validate
the optimal mass of feathers for determining CORT content, we assayed four different masses
of feathers that were pooled from three individuals: 12, 25, 50 and 75 mg, with four replicates
each. This showed that a minimum of 25mg of feathers was needed to overcome excessive
intra-assay variability (see results). Because we were restricted in the number of feathers we
could collect from free-living birds, we had to pool samples from three individuals (of the
same sex and weight) to get 35mg of feathers for the CORT assay. We restricted feather
sampling to adults, as juveniles grow their feathers on the breeding ground.

(2) Parasite screening

A total of 363 individual turnstones were screened for haemosporidian parasite infection, 14
of which were sampled in Broome, 247 from South Australia and 102 from King Island.
DNA was extracted from blood samples using ammonium acetate/ethanol precipitation (Richardson et al. 2001) and samples were molecularly sexed following Griffiths et al. (1998) to confirm extraction quality. Samples were screened for haemosporidian DNA (Plasmodium or Haemoproteus spp.) using a nested PCR technique, following the protocols in Waldenström et al. (2004). Given the low prevalence for haemosporidian infections previously found in shorebird species (Mendes et al. 2005a), numerous positive controls were included in each reaction and all samples were screened twice to ensure false negatives were minimised.

(3) Fuel deposition rates

Fuel deposition rates (FDR, expressed as body mass gain in g/d⁻¹) were estimated from the correlation we established between FDR and latitude of shorebird staging areas (Chapter 3). The details and background of the model are specified in Chapter 3. Briefly, we used published data on FDR in migratory shorebirds and, after correcting for lean body mass (Lindström 2003), we found a significant correlation between FDR and absolute latitude. Although metabolised energy intake is considered the most important determinant of fuelling rates, variations in daily energy expenditure play an important role in modifying fuelling rates. We therefore also estimated daily energy expenditure (DEE) following Wiersma and Piersma (1994) and Piersma (2002). Monthly mean temperature, solar radiation and wind speed at each site were downloaded from (http://climexp.knmi.nl/).

Statistical methods

Many of the feather samples had very low levels of CORT and were therefore at the end of the linear part of the standard curve (29 out of 42 samples from South Australia and 103 out of 130 samples from King Island). This meant that although we were confident of the relative ranking of the samples, we were not confident of their actual feather CORT concentration. We therefore used the non-parametric Kruskal-Wallis rank sum test to examine differences in feather CORT levels between populations. We then used linear models to test the effect of body mass as an explanatory variable on feather CORT concentrations.

We used linear models to examine the effect of site (Broome, SA or KI), sex and weight on leukocyte concentrations. Leukocyte data were log transformed for normality, except in the cases of basophils and monocytes, where we used the non-parametric Kruskal-Wallis test as there were many zero values. We also included the time between capture and sampling as a
covariate in all models, because handling time has been found to affect leukocyte concentrations in shorebirds (Buehler et al. 2008). Covariates were removed from models where \(P > 0.05\).

All statistical analyses were conducted using R version 3.0.3 (R Core Team 2014)

**Results**

*CORT levels in feathers*

In line with findings of Lattin et al (2011), we found that the calculated concentration of CORT/mg feather was dependent on the mass of the original sample used, with variation between replicates being much higher in the smaller mass samples than the larger ones (Fig.2). We therefore used 35mg of feathers for determining feather CORT of free-living birds, which was a compromise between feather availability and assay robustness. When comparing the two populations (SA and KI), we found significantly higher CORT content in feathers of birds from SA (\(\chi^2=6.26, \text{df}=1, p=0.012\)) (Fig.3). Body mass did not explain variation in CORT content.

![Figure 2](image.png)

*Figure 2:* The relationship between feather corticosterone (cort,) concentration and sample mass (mg) in samples of pooled pulverized feathers from three individual ruddy turnstones (in different colors). Four replicates for each of the four pooled-feather masses are represented.

*Leukocyte concentration (circulating cellular immunity)*

There was a significant effect of time from capturing until sampling on H/L ratio (\(\beta=0.88, p<0.01\)) and eosinophil number (\(\beta=-0.34, p<0.01\)). We therefore corrected H/L ratios and eosinophil number in relation to time since capture. H/L ratio was significantly lower in birds
from SA than in birds from Broome ($\beta=-0.47, p=0.05$), but no significant difference was found between birds from Broome to birds from KI. We also found significantly lower H/L ratio in males than females ($\beta=-0.7, p<0.01$).

Birds from Broome also had significantly fewer monocytes than those in SA ($\chi^2=24.15$, df =1, $p<0.01$) and KI ($\chi^2=20.62$, df =1, $p<0.01$). Eosinophil number was significantly higher only in birds from KI than birds from Broome ($\beta=0.32, P=0.03$). Basophil number was significantly higher in birds from SA than in birds from KI and Broome ($\chi^2=36$, df =2, $p<0.01$).

**Figure 3**: (A-D) Stress biomarkers (leukocyte concentration and feather CORT levels), (E) expected fuelling rates measured as mass gain per day (FDR) and (F) body weight for individuals in the three wintering sites Broome, South Australia (SA) and King Island, Tasmania (KI). Feathers were not collected in Broome, therefore data are not available. FDR were estimated based on the correlation we established between FDR and latitude of shorebird staging areas (see details in text and chapter 3).

**Blood parasite infection**
We identified 14 infected individuals out of the 363 sampled, indicating an overall prevalence of 3.86%. Among sampling sites, infection prevalence varied considerably, with higher prevalence exhibited in Broome (3 infected out of 14 total; 21.0%) compared to SA (7 infected out of 247 total; 2.8%) and KI (4 infected out of 102 total; 3.8%). The low sample size for Broome, however, prevented a powerful statistical comparison of infection proportions among sample sites (power < 0.5).

*Fuel deposition rates*

Expected fuel deposition rates were higher on KI and in SA (1.24 and 1.16 g/d\(^1\), respectively) than Broome (0.67 g/d\(^1\)). DEE was lower in Broome than in SA and KI due to the higher temperatures and the lower wind speed (432.8 and 305.6 kj/d\(^1\) on average on King Island and in SA, respectively, and 143.9kj/d\(^1\) on average in Broome). Also, body mass in Broome was significantly lower than in SA and KI (Fig.3). All parameters are also summarized in Appendix S1.

**Discussion**

Our findings represent three lines of evidence to contribute to the hypothesis that ruddy turnstomes fly the “extra mile” to improve their wintering conditions in Australia. These lines of evidence do not directly support the alternative hypothesis that these are the better quality birds that fly further. Firstly, the different white blood cell profiles from the three sampling sites suggest that wintering birds in Broome exhibit higher stress levels compared to birds wintering at more southern latitudes. The combination of high H/L ratio and low counts of eosinophils and monocytes found in birds from Broome (although count of eosinophils at SA was not significantly higher than the Broome count) further support the conclusion that this change in leukocyte profile is a stress reaction rather than a response to disease (Jain 1986). However, in both cases (stress or disease), our results suggest poorer health condition in the Broome population, in comparison to birds from SA and KI. Although the function of basophils is still poorly understood, Basophilia (i.e. a relatively high number of basophils) may be a unique physiological response in birds exposed to severe stress (Maxwell & Robertson 1995). Although we only recorded basophilia in SA, we cannot entirely exclude the confounding effects of high humidity in Broome on these results; basophils are extremely water soluble (Cotter, 2001), therefore the cells might have been damaged in the process of
making the smears under the humid environment of Broome. Indeed, the blood smears from Broome had many fragmented cells, more than in SA and Tasmania.

The gender differences in H/L ratios that we found may be related to dominance interactions and access to resources, but may also represent some inherent differences between the sexes in haematological characteristics and immunity (Plischke et al. 2010).

The second line of evidence is the trend for higher parasite prevalence in wintering turnstones in Broome compared to their SA and KI counterparts. This trend suggests that turnstone populations wintering at higher latitudes in Australia may be encountering lower pressures from haemosporidian blood parasites, although more data is needed to determine if this pattern is statistically robust. While our sample size for Broome was particularly low (N = 14 individuals) and prevented statistical comparison among sites, the latitudinal pattern of prevalence is similarly exhibited across a wider range of wintering shorebird species in Australia (Clark et al. in prep), Africa and Europe (Mendes et al. 2005b) as well as in tundra swans *Cygnus columbianus* in north America (Ramey et al. 2012). Latitudinal variation in haemosporidian prevalence is not unexpected since vector activity and within-vector parasite development are both positively related to temperature (Santiago-Alarcon et al. 2012). Such variation in prevalence among wintering sites could therefore contribute to the incentive to ‘fly the extra mile’ for some wintering shorebird populations, particularly if infections are harmful to host fitness. For instance, because long-distance migrations require sustained aerobic capacities, decreases in vitality associated with parasite infections may have large negative consequences for migrants (Klaassen et al. 2012). Infection with avian haemosporidians has been linked to poor body condition and delayed arrival time in migratory species (Møller et al. 2004, Garvin et al. 2006), which could be a consequence of hampered foraging ability prior to departure (Van Gils et al. 2007). For migratory shorebirds, fitness consequences of infections picked up during the winter could result in a hampered ability to complete the migration north to breeding sites (D'Amico and Baker 2010, Mendes et al. 2013). While we can only speculate on the fitness consequences of haemosporidian infections in ruddy turnstones, the identification of infections in all three sampling sites in this study corroborate the suggestion that haemosporidians are a good model system to investigate the effects of parasitism on migration pathways in migratory shorebirds (Piersma 1997, Mendes et al. 2005a).
Finally, our modelling results suggest that predicted fuel deposition rates are lower in Broome than in SA and KI, which are located at higher latitude. Metabolised energy intake is considered the most important determinant of fuelling rates (Lindström 2003), and variation in fuelling rates between sites are thus assumed to be closely linked to local differences in food availability (e.g. Piersma et al. 2005, Van Gils et al. 2005). The predicted higher energy expenditure rates for birds on KI and in SA further add to this result, as it shows that much of the energy that might have been directed to fuel storage was instead used to meet higher thermoregulatory costs. Despite this additional expenditure, birds on KI and in SA manage to gain higher mass per day. Several field observations in the area of Broome suggest that ruddy turnstones may feed on non-preferred food items. For example, ruddy turnstones were observed foraging for sustained periods by flipping over brittle stars, probably to feed on their gonads (Rogers 2006) or by flipping Sand-dollars (Bornmann 1985). These observations suggest that prey are not abundant in the Broome area. By contrast, turnstones in SA and KI, were consistently observed fossicking in beach wrack and pulling out maggots and other small invertebrates, which are present in high densities in washed-ashore kelp originating from the large offshore kelp beds (YAR, pers. observ, Rogers D, pers. comm). Orr (2013) found a strong positive relationship between wader abundance and the percentage cover of wrack on beaches. Similarly, Dugan et al. (2003) reported a positive correlation between actively feeding shorebirds and density of wrack in southern California. Moreover, they show that beaches that accumulate wracks had a greater diversity of trophic links and more functional redundancy, making their food webs more resilient to perturbations. These observations support our modelling results showing that intake rates are higher in SA and KI rather than in Broome. The low body mass found in birds from Broome relative to SA and KI was expected, as in general, shorebirds in the tropics are leaner and have lower basal metabolic rates (BMR) (Kersten et al. 1998).

Our feather CORT results provide further evidence for an added advantage for birds that elect to fly an additional 500 km south. Although some caution is needed when interpreting CORT levels in feather (see chapter 6), the lower CORT concentration in feathers of birds from King Island might be due to better feeding conditions, also presented by our modelling showing higher fuelling rates in higher latitude. Another possibility is that the low human population density on KI contributed to lower disturbance levels and therefore to lower stress level. Overall, a large number of the samples had very low levels of CORT in both populations. A reference value of CORT in feathers in this species does not exist, we therefore can only
speculate whether the low CORT levels indicate a general low level of stress in both population, or represent a “normal” level of CORT in feathers of ruddy turnstone.

We also confirmed two fundamental issues in the use of feather CORT and the importance of determining optimal feather mass for good quality data. First, our results demonstrate that variation between replicates was strongly affected by the mass of feathers in each sample. Second, we confirmed that feather CORT concentration was reduced with increasing sample mass. These data demonstrate the fundamental importance of testing the appropriate sample size for CORT extraction from feathers, before embarking on a sampling protocol.

There are additional factors potentially contributing to the higher stress levels of birds from Broome than birds from SA and KI. For example, birds in the tropics are exposed to greater heat stress than temperate residents which may result in higher H/L ratios (e.g. Altan et al. 2003). Also, shorebirds are often disturbed by birds of prey in the area of Broome (e.g., by White-bellied Sea Eagle *Haliaeetus leucogaster*, Black Kite *Milvus migrans* and Brahminy Kite *Haliastur indus*). Attacks are not so common, but the appearance of these predators causes regular flushing of birds from the beach (C. Hassell, pers.comm) and may increase their vigilance activity at the expense of feeding. In contrast, in South Australia and King Island, birds of prey are not as common and include mainly Peregrine Falcon *Falco peregrinus* and Australian Hobby *Falco longipennis*, and attacks on shorebirds are rare (C. Minton, pers.comm.).

In high Arctic breeding shorebirds, breeding success is highly correlated with arrival date, and we would therefore expect some benefit in wintering closer to the breeding ground (Broome in our study). Our data are consistent with the interpretation that birds occupying more costly wintering locations in terms of higher migratory flight and thermoregulatory costs, as in South Australia and King Island, are compensated by better feeding conditions and lower blood parasite infections, which facilitates timely and speedy migration back to the breeding ground. It is therefore suggested that the overall benefits of the more distant wintering locations are sufficiently positive to outweigh the substantial additional migration costs. These data contribute to our understanding of cost/benefit trade-offs in the decision making underlying migratory behaviour.
Acknowledgements

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Appendix

Appendix S1

Table A1: Summary of parameters measured in ruddy turnstones in three wintering sites (range is presented where applicable). Averages and standard deviation are in brackets.

<table>
<thead>
<tr>
<th></th>
<th>King Island</th>
<th>South Australia</th>
<th>Broome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males/females</td>
<td>23/27</td>
<td>21/19</td>
<td>NA</td>
</tr>
<tr>
<td>Mean mass (g)</td>
<td>155.9 (+16.1)</td>
<td>152.7 (+16.4)</td>
<td>105.9 (+10.7)</td>
</tr>
<tr>
<td>H/L ratio</td>
<td>0.09-10.7 (1.9±2.1)</td>
<td>0.03-17.2 (1.8±2.7)</td>
<td>0.3-15.5 (4.9±3.8)</td>
</tr>
<tr>
<td>% eosinophils</td>
<td>0-23 (8.8±4.9)</td>
<td>0-23.6 (7.2±5.4)</td>
<td>0-17 (5.2±5.1)</td>
</tr>
<tr>
<td>% monocytes</td>
<td>0-14 (4.4±3.1)</td>
<td>0-18.5 (5.7±4.3)</td>
<td>0-4 (1.1±1.2)</td>
</tr>
<tr>
<td>% basophils</td>
<td>0-11.6 (2.4±2.9)</td>
<td>1-25.9 (7.3±5.5)</td>
<td>0-8 (1.5±2.2)</td>
</tr>
<tr>
<td>Potential fuel deposition rate (g/day⁻¹)</td>
<td>2.3</td>
<td>2.2</td>
<td>1.3</td>
</tr>
<tr>
<td>Daily energy expenditure in the months March-May (kj/d⁻¹)</td>
<td>398.1-471.6 (432.8)</td>
<td>278.5-338 (305.6)</td>
<td>143.9</td>
</tr>
<tr>
<td>Corticosterone (pg/mg feather)</td>
<td>2.3-161.3 (28.64±29.39)</td>
<td>9-136 (48.67±34.33)</td>
<td>NA</td>
</tr>
</tbody>
</table>
Chapter 6

An experimental examination of the relation between plasma and feather corticosterone content in the House Sparrow, *Passer domesticus* in southeast Australia

Yaara Aharon-Rotman, Katherine L. Buchanan, Marcel Klaassen and William A. Buttemer

5 Author contributions: WAB and YAR originally formulated the idea and performed the experiment. WAB, KLB and YAR performed lab work. YAR and MK performed statistical analysis. YAR wrote the manuscript with advices from all other authors.
Abstract

Non-invasive techniques for measuring glucocorticoids (GCs) are gaining favour, largely due to the advantage of eliminating the effects of animal disturbance on the GC levels being measured. In the case of birds, GCs are deposited in feathers as corticosterone (CORT), which provides a further advantage in reflecting a bird’s GC status over their period of feather synthesis. This thus permits a retrospective view of the average circulating GC levels during the moult period. Despite the increasing popularity of this technique, very few studies have experimentally tested the reliability of feathers to reflect plasma corticosterone (CORT) levels during moult or the ability to discern the chronology of stress during moult by analysing the CORT content of sequential sections of a feather. We therefore validated the use of CORT to estimate plasma CORT levels in house sparrows, Passer domesticus. Plasma levels of CORT were experimentally manipulated by implanting each bird with either a corticosterone-filled, metyrapone-filled, or empty (‘sham’) silastic capsule prior to regrowing plucked feathers. We also scored the appearance of fault bars on the replacement feathers of these birds. In line with our expectations, and after correcting for sex, CORT of feathers plucked prior to experimental manipulation and site of capture, the CORT of birds treated with CORT implants was higher than that of the sham group. Although the metyrapone-treated group was expected to show low levels of CORT due to its inhibitory effect on GC synthesis, CORT of these birds did not differ from the CORT group and were higher than levels measured in the sham group. By contrast, feather fault bar scores were significantly higher in CORT treated birds than in the other treatment groups, consistent with the known effects of exogenous CORT in compromising feather quality. Our study therefore broadly confirms the use of feathers as a non-invasive tool to assess plasma CORT in birds, but importantly demonstrates and highlights the importance of accounting for inter-individual variation before interpretation of plasma hormone levels of moultling birds from analysis of CORT alone.
Introduction

In dealing with a changing environment, an animal’s stress response is a critical element in retaining its well-being. A stress response is a suite of physiological and behavioural coping mechanisms which occur when an organism confronts challenges to its homeostasis (Selye 1973) and may include prioritising allocation of its resources towards self-maintenance functions (Jenni et al. 2000). When a vertebrate responds to a stressor, its hypothalamic-pituitary-adrenal (HPA) axis is activated and releases glucocorticoids (GC) from the adrenal cortical tissues within minutes for up to several hours (Sapolsky et al. 2000). This mechanism redirects an animal from its normal activities to a ‘life-saving’ or ‘emergency’ state (Wingfield et al. 1998), which facilitates their ability to overcome stress and restore homeostasis (Wingfield and Romero 2001, Charmandari et al. 2005, Bortolotti et al. 2008).

The stress response works well in dealing with acute environmental challenges. Under these conditions, GC hormones are secreted in proportion to the extent and duration of the stress and then return to baseline levels via negative feedback signals (Wingfield and Sapolsky 2003). However, when exposure to stressors is chronic and the HPA axis response is overstimulated (Romero 2004), feedback signals are weakened and GC secretion persists for extended periods (Boonstra et al. 1998, Romero 2004). Chronically elevated levels of GC hormones (cortisol and corticosterone) may have detrimental health consequences. These include declines in immune function, body condition, growth, reproduction and survival (Boonstra et al. 1998, Sapolsky et al. 2000, Charmandari et al. 2005, Blas et al. 2007). Increased glucocorticoid levels are also associated with greater susceptibility to parasites, and may activate latent infections due to their suppressing effects on the immune system (Gylfe et al. 2000, Mougeot et al. 2010).

It is challenging to distinguish acute stress from chronic stress from analysis of an animal’s plasma GC content. Firstly, serial sampling over several days would be required to determine the persistence of elevated GC levels (Sheriff et al. 2011), something that is very difficult to achieve when studying free-living animals. Secondly, the process of capturing and handling an animal when obtaining blood samples provokes a stress response, which starts within minutes of an animal first being disturbed. Consequently, obtaining baseline plasma GC in endothermic vertebrates requires blood to be collected within 2 to 5 minutes of initial disturbance (Romero and Reed 2005). These difficulties in quantifying the extent of chronic
stress in free-living animals have provoked development of a number of alternate sampling techniques, particularly non-invasive methods.

There has been an increasing use of non-invasive techniques for determining an animal’s GC status. These include determination of GC content in an individual’s hair, feathers, or its excreta (Goymann 2005, Sheriff et al. 2011). The use of such non-invasive techniques eliminates the effects of animal disturbance on the GC levels being measured. A further advantage in the case of examining GC content of fur or feathers is that these accumulate GC over their period of their synthesis, thus permitting a retrospective view of the average GC levels over their period of moult. This is especially advantageous for revealing the stress history of migratory species while moulting, which often takes place in inaccessible or unknown locations. Although there is great potential in using these techniques to assess long-term stress levels, validation of these techniques in relation to plasma GC levels is essential (Buchanan and Goldsmith 2004).

During avian moult, corticosterone (CORT) and its metabolites are deposited into developing feathers and are believed to reflect plasma levels on the day that part of the feather was being synthesized (Bortolotti et al. 2008, Bortolotti et al. 2009). If this assumption is valid, CORT levels in feathers will represent an integrated average of plasma CORT levels over the period of that feather’s maturation. The reliability of this method to reflect stress status during moult and, further, the ability to discern the chronology of plasma CORT during moult from analysing sequential sections of a feather have seldom been validated. Those that have been done confirm that experimentally increased plasma CORT does result in increased feather CORT (CORTf) both in the European starling *Sturnus vulgaris* (Lattin et al. 2011) and in tree swallows *Tachycineta bicolor* (Fairhurst et al. 2013). In validating this method, several notes of caution have been raised. Firstly, it was found that antibodies with high specificity to CORT were unable to detect CORT in feathers, but a CORT antibody with moderate cross-reactivity to other steroids was successful (Lattin et al. 2011). This suggests that the GC in feathers may be CORT metabolites and thus calls into question the level of correspondence between plasma and feather CORT contents. Secondly, feather CORT has been found to vary markedly in relation to the mass of sample being analysed. Using pooled samples of starling feathers (Lattin et al. 2011) and ruddy turnstones (Aharon-Rotman et al. unpubl. data and chapter 5), both studies showed that CORTf decreased with increasing sample mass, but, more importantly, variation in CORTf between replicate samples also decreased substantially.
Thus, it is very important to establish the appropriate minimum sample mass needed when evaluating CORT$\text{f}$ in a given species.

Feather quality is also known to reflect the health status of moulting birds (e.g. DesRochers et al. 2009) and, consequently, has the potential to serve as a biomarker for stress while moulting. CORT stimulates protein breakdown and, as feathers are mainly keratin proteins, high plasma CORT can have negative effects on feather growth and structure (Strochlic and Romero 2008, DesRochers et al. 2009). Fault bars, which are structural defects produced under stressful and adverse conditions (Riddle 1908), appear at higher frequency when birds are malnourished (Murphy and King 1982) or under greater predation risk (Witter and Lee 1995). There is evidence that feather quality correlates directly with fitness (Bortolotti et al. 2002), with birds having a greater number of fault bars suffering lower reproductive success and reduced longevity. Thus, fault bars should provide an indication of a birds stress status during moult.

Our objective in this study is to further validate the use of feathers to estimate CORT levels in birds. Using experimental manipulations of plasma CORT levels, we examined the effect of plasma CORT manipulation on both CORT$\text{f}$ and the extent of fault bars on feathers synthesized during these treatments.

**Methods**

House sparrows Passer domesticus were captured at Torquay, Victoria in summer 2012 and at Werribee, Victoria in autumn 2013. After capture, sparrows were placed 2 per cage (ca. 40 x 60 x 60 cm) and held in a room with temperature maintained at 25$^\circ$C and photoperiod set to 14L:10D. Commercial finch seed mix, grit and water were provided *ad libitum*. After at least 1 week of adjustment to captivity, we removed birds from their cages, anaesthetized them with methoxyflurane before plucking 12 feathers from their wings and tail (five primaries, five secondaries and two rectrices), to be used as a reference for individual pre-treatment CORT$\text{f}$ values. After recovery from anaesthetic, birds remained in their holding cages until feather regrowth was noted (14 and 12 days after plucking in the Torquay and Werribee population, respectively). We then manipulated corticosterone levels by implanting each bird with either a corticosterone-filled (Sigma-Aldrich, Sydney, New South Wales, Australia; catalogue number 27840), metyrapone-filled (Sigma-Aldrich; catalogue number M2696), or empty silastic capsule (referred to as “sham” in this paper). We used metyrapone-filled
implants to reduce plasma CORT content as it is known to reduce glucocorticoid synthesis by its inhibition of steroid 11β-hydroxylase (Drouet et al. 2010). The capsules were prepared from 10 mm lengths of medical-grade silastic tubing (1.47 mm inner diameter; 1.96 mm outer diameter; Dow Corning, Sydney, New South Wales, Australia), which were sealed at each end with silastic glue in metyrapone and control capsules and at one end in those filled with corticosterone. Capsules were individually implanted subcutaneously along the left flank beneath each wing under anaesthesia (methoxyflurane), and the incision was closed with suture and medical-grade cyanoacrylate adhesive (Vetbond). The efficacy of the hormone implants was validated using a different set of birds (see validation of implants below). A day after receiving implants, birds were transferred from their holding cages to outdoor flight cages (ca. 2 x 3 x 2.5 metres) until replacement feathers were fully regrown (21 and 28 days in the Torquay and Werribee population, respectively). These outdoor cages contained dense foliage and sheltered perching areas to provide birds with diurnal and nocturnal refugia, respectively. After the plucked feathers were fully regrown, birds were removed from the flight cages, weighed, and the replacement feathers then plucked and stored in labelled paper envelopes at room temperature until later analysed.

After feather plucking, we examined all feathers and scored the presence of fault bars using the following scoring criteria:

1. Translucent bar present, but barbules intact (probably lack of melanin).
2. Some damage to barbules, but no obvious gap
3. Missing barbules, forming a gap
4. Prominent damage to barbules, and malformed
5. Feather shaft broken

For each individual, we scored each of the 12 initially plucked feathers and those that regrew after getting an implant to obtain an individual cumulative fault bar score pre and post implants, based on the above criteria.

We selected 5 of the plucked feathers from each bird and pooled these to determine their average CORT. The remaining 7 feathers were sent to a laboratory in Canada for a parallel study to characterise the GC content in the feathers using mass spectrometry.

_CORT assay protocol_
Feathers were prepared and assayed following Lattin et al. (2011). Briefly, after removing the calamus, the 5 feathers from each bird were cut into small pieces <5mm$^2$, mixed thoroughly, from which a 30mg sample was placed into a 25 mm flask. This feather mass was previously validated to be the minimum amount needed to get statistically repeatable results (Lattin et al. 2011, Aharon-Rotman et al. unpubl.data and chapter 5). We then added 7ml of methanol to each sample and placed the covered flask in a sonicating bath for 30 minutes. Samples were then placed on a vortex platform (Ratek Instruments, Boronia, Australia), which was housed in an incubator that was maintained at 50°C overnight. The liquid fraction of each tube was separated using vacuum filtration (Filtech, filter paper #4, catalogue number 1893-055) and placed in a 100 x 13mm culture tube and then dried under nitrogen gas on a heater block at 50°C. The dried residue was reconstituted in Tris-HCl buffer and run through a standard radioimmunoassay (RIA) using a corticosterone antibody (Sigma-Aldrich, Saint Louis, Missouri, USA, product No. C8784). Samples were analysed in 2 assays and the mean 50% binding level was 1.38 ng/ml. Samples were initially spiked with ca. 1 pg of tritiated corticosterone (1,2,6,7-3H; Perkin Elmer, Australia) prior to steroid extraction to determine percentage recovery. Average CORT recovery in reconstituted samples was 82% (range 65-94%). All CORT values were adjusted for each individual’s recovery percentage. Some samples had very low levels of CORT, which were beyond the linear part of the standard curve (4 out of 94 samples from Torquay and 23 out of 90 samples from Werribee). Although we are confident that these samples were of lower ranking than those within the linear portion of the calibration curve, we cannot ascribe absolute CORT levels to them. Therefore, all samples with binding levels > 83% were set to 30pg CORT/mg feather, which was the lowest detectable value on the linear curve.

Inter and intra-assay variation

In order to quantify inter-assay variation, we created three homogenous mixtures of feathers of ruddy turnstone, Arenaria interpres that were each pooled from carcases of three different individuals and assessed in duplicate in each assay. Feathers were cut in small pieces (<2mm) and were pulverized in a ball mill (QIAGEN GmbH, Hilden, Germany). Samples from each of these pools were run in each assay to determine inter-assay variation.

Coefficient of variation (%CV) was calculated as the standard deviation (SD) of the mean × 100 / mean. The intra-assay CV was 8.4% and 10.1% for the Torquay and Werribee samples, respectively. Inter-assay variation was 7.4%.
Validation of Implants

15 house sparrows were captured in Torquay prior to moult in November 2012 and held for 2 weeks in captivity (details of holding room are as described for sparrows used for this study prior to plucking). Sparrows were then divided into 3 groups of 5 birds each:

1) CORT treatment
2) Metyrapone treatment
3) Sham treatment (empty implant)

Treatments were randomly assigned to the birds and implants were inserted into 2 birds per day to allow a staggered sampling regime (Surgical procedures is the same as in main experiment and detailed above). When sampled, 2 birds were removed from their shared cage and had a 120 μl blood sample collected within 3 minutes of first being disturbed. The CORT and metyrapone-treated birds were sampled at 2, 5, 10 and 15 days after receiving implants, whereas the control birds were sampled 5 and 10 days after implantation.

The corticosterone content of plasma samples was determined in triplicate using an EIA kit (Enzo Life Sciences; catalogue number ADI 900-097). Samples were initially spiked with ca. 1 pg of tritiated corticosterone (1,2,6,7-3H; Perkin Elmer, Australia) prior to steroid extraction to determine percentage recovery. After extraction in dichloromethane, samples were dried under nitrogen and then reconstituted in the manufacturer’s buffer solution (1:30 ratio). Hormone values were adjusted for individual sample recovery, which averaged 92.2 ± 2.1%. Reported hormone values are based on the average of the two closest of the triplicate values, except in cases where these had >10% variation. In such instances, remaining plasma for these samples was reanalyzed in an additional assay. Intra-assay variation using triplicate standards and samples was 4.2%.

Statistical methods

We used a linear model when analysing CORTf (pg/mg feather, post implants) as the dependent variable in relation to treatment (CORT, sham or metyrapone), reference CORTf (CORTf prior to implants), body mass, sex and the original catching site of the birds (Torquay or Werribee). Reference CORTf was entered as a covariate as we expected individuals with high pre-treatment CORTf would likely also have higher CORTf post treatment. Body mass was entered as a covariate in the model as an indicator of bird
condition. We expected high body mass to reflect better feeding conditions and thus lower CORT$_f$ levels. Since CORT levels are known to differ between sexes, we also considered sex in the model. Finally, since we used birds originating from two different sites that may vary in their exposure to stress (e.g. variation in predation pressure and disturbance) we also used the origin of the birds as a factor in the model. The initial model contained the main effects only after which we entered all possible interaction terms, one by one in a forward, stepwise approach, to derive at the final model with the lowest AIC. To evaluate differences between groups (i.e. treatments, sex and sites) we used TukeyHSD post hoc tests.

We used ordinal logistic regression (i.e. ‘proportional odds model’) to analyse fault bars as the dependent variable using treatment type (CORT, sham or metyrapone), body mass, sex, the original catching site of the birds (Torquay and Werribee) and CORT$_f$ post treatment as explanatory variables (i.e. the same variables as in the CORT$_f$ analysis). Fault bar scores were categorized into four levels corresponding to the sum of fault bar scores of 0-5, 6-10, 11-15, and >15. For each parameter in the final model we calculated the odds ratio (OR), which is the expected increase in fault bar scores on the log odds scale for every unit of increase in the predictor variable (while holding all the other variables in the model at a fixed value), and its 95% confidence interval. An OR>1 indicates a positive, whereas an OR<1 indicates a negative effect of the predictor variable.

We modelled the location of fault bars on the feather from the tip in relation to treatment type (CORT, sham or metyrapone), body mass, sex, the original catching site of the birds (Torquay and Werribee) and CORT$_f$ post treatment using linear model. We expected that the timing of appearance of fault bars will reflect the period during which plasma CORT was high.

To test the distribution of fault bars across feather orders (wing and tail) we again used ordinal logistic regression. Fault bar scores of each individual feather (in a scale of 0-5) were modelled as the response variable and feather order (Wing and tail) as the explanatory variable.

CORT prior and post treatments, as well as distance of fault bars from feather tip were log-transformed in all models to guarantee homogeneity of variances. Variables were excluded from linear models using ANOVA type III, based on significant level (set to p=0.05). All statistical analyses were conducted using R version 3.0.3, R Development Core Team (2014).


Results

**Implant validation**

Validation of the implants showed that over a 15-day test period, plasma CORT in the CORT-treated birds was higher than in either control or metyrapone-treated birds. Plasma CORT in the CORT-treated birds was highest in the second day post implant, then decreased but remained high until the end of the experiment (Fig. 1). Plasma CORT levels in the metyrapone-treated birds were similar to those in sham-treated birds 5 days after implantation and increased with time, having the highest levels 10 days after receiving implants (Fig. 1). Sham implanted birds had the lowest plasma CORT levels of all treatments, averaging 25.3±5.3 and 17.2±2.1 ng/ml⁻¹, 5 and 10 days post implant, respectively (Fig.1). Importantly, the metyrapone implants were exhausted by the end of the 15-day trial, while the CORT-filled implants still contained CORT. Based on this validation, we expected the replacement feathers of CORT-treated birds to have the highest CORT, followed by those from metyrapone-treated birds, and for sham-treated birds to have the lowest CORT. Results from the validation experiment also suggested that fault bars in the metyrapone-treated birds would appear later (i.e. further from the feather tip) as CORT levels increased only after about 10 days of implantation (Fig. 1). In the CORT-treated birds, we expected fault bars to appear closer to the feather tip due to the immediately higher levels of plasma CORT following implantation.

**Figure 1:** Plasma CORT levels (ng ml⁻¹) in the implant validation study for birds treated with corticosterone (CORT), metyrapone or empty (sham) implants. Plasma CORT was measured 4 times during the 15-day experiment (twice in the sham treatment), depicted on the x-axis.
CORTf post implants

None of the interactions significantly improved the model and the final model therefore included only the main factors. After accounting for sex, body mass, site of origin and reference CORTf, we found a significant effect of treatment on CORTf (F2,82=7.77, P<0.001). The sham treatment produced the lowest CORTf values, whereas metyrapone and CORT treatments were higher, but statistically indistinguishable from one another (Fig. 2). A post hoc Tukey HSD test revealed that the differences were significant only between the sham and the metyrapone treatment (P<0.001) and the sham and the CORT treatment (P=0.03)

Individual levels of CORTf prior to the experiment had a significant positive effect on their CORTf content post treatment (F1,82=38.9, P<0.001). Females had higher CORTf than males (F1,82=4.62, P=0.03) and individuals from Werribee had higher CORTf than individuals from Torquay (F1,82=76.48, P<0.001). Body mass had no significant effect on CORTf (F1,82=0.79, P=0.38). One bird had exceptionally high levels of CORTf (446.3 pg/mg). We did not exclude this outlier from our initial analysis, but when the model was run without the outlier the same statistical outcomes were obtained, thus confirming the robustness of these results.

![Figure 2](image)

**Figure 2**: Partial residuals of the predictors (A) treatment (metyrapone [mety], sham or CORT (B) CORTf prior to experiment (pre-treatment CORTf levels (C) site of origin and (D) sex, in relation to CORTf post treatments. In panel A, groups with the same letter are not significantly different from each other (Tukey HSD test, P>0.05). In panels C and D groups are statistically different from each other (Tukey HSD test, P<0.05)
Fault bars

In both populations, naturally moulted feathers (prior to implants) rarely had fault bars (five individuals out of the 23 individuals that were scored from Torquay had an average fault bar score of 5.6, and six individuals out of 23 from Werribee had an average score of 2.8).

There was a significant effect of treatment on fault bar scores. Although the odds of having fault bars of a high score in the metyrapone treatment were similar to the odds for the same high score fault bars in the sham treatment (i.e. 95% confidence interval includes 1), the odds of identifying fault bars of the highest score category are 28 times higher in the CORT treatment group than the odds for the same effect in the sham treatment (Fig. 3, table 1). We also found a significant difference between the sexes and between sites with odds for appearance of high score fault bars in males 9 times the odds for females and 5 times in Torquay than Werribee. We did not find an effect of body mass or levels of CORT$_T$ (for both 95% confidence interval included 1; table 1) on individual fault bar scores.

The location of fault bars on the feather (distance from the feather tip) was significantly different between sites, with fault bars of feathers of the Torquay population being closer to the feather tip ($F_{1,72}=8.23$, $P=0.005$). There was a significant difference between treatments as well ($F_{2,72}=4.33$, $P=0.02$), with post hoc testing revealed that fault bars on sham treated birds were closer to the feather tip than fault bars in the metyrapone treatment ($P=0.044$) and in the CORT treatments ($P=0.028$) (Fig. 4).
Figure 4: Partial residuals for the effect of (A) treatment (metyrapone [mety], sham and corticosterone [cort]) and (B) site of origin in relation to location of fault bars on feathers (distance from the feather tip). In panel A, groups with the same letter are not significantly different from each other (Tukey HSD test, P>0.05). In panel B, there is a significant difference between the two sites (Tukey HSD test, P<0.05).

Moreover, there was a significant difference between fault bar scores in relation to the different feather orders (Fig. 5). The odds of having a high fault bar score were 3 times higher in tail than in wing feathers (table 1).

Figure 5: Average fault bar scores on tail feathers (rectrices, N= 90) against average fault bar scores on wing feathers (N=435). Wing feathers include both primaries and secondaries. Odd ratios are presented in Table 1.
<table>
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<tr>
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<tr>
<td>Tail feathers</td>
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<td>1.94</td>
<td>4.64</td>
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</table>

**Table 1:** Odd ratios (OR) for the best ordinal logistic model for (A) cumulative fault bar scores per individual bird post treatments in relation to the treatment given, sex, site of origin, body mass and reference feather CORT (CORT<sub>f</sub> levels measured prior to implantation) and (B) average fault bar score in relation to feather order (tail or wing). Lower and upper 95% confidence interval (CI) are depicted. If the CI included 1, the effect is non-significant.

**Discussion**

The theory that CORT<sub>f</sub> reflects the integrated stress levels of birds at the time of moult assumes there to be a strong correspondence between plasma and feather CORT levels. There is further assumption that any variance in CORT<sub>f</sub> along the length of a given feather directly reflects plasma CORT levels at the time that section of feather first emerged (Bortolotti et al. 2008, Fairhurst et al. 2011).

We found CORT-treated birds to have significantly higher CORT<sub>f</sub> than sham-treated individuals, thus confirming the experimental results of Lattin et al. (2011) and Fairhurst et al. (2013). The lack of difference between CORT<sub>f</sub> in metyrapone and CORT-treated birds was unexpected, but may be explained by the longevity of the metyrapone implants. Our validation study of the implants on a different set of birds showed that plasma CORT in birds treated with metyrapone implants were initially much lower than CORT-treated birds, but increased to similar high levels 10-days post implant when the implants were exhausted. A rapid increase in plasma CORT after a CORT suppression period by the metyrapone may explain the generally high CORT<sub>f</sub> found for the metyrapone-treated birds, as the CORT<sub>f</sub>
represents an average CORT level over the feather growth period. We did not sample plasma CORT in birds used for the feather-regrowth study to avoid confounding the experiment with the stress effects of serially sampling birds. Consequently, we have no direct indication of the efficacy and longevity of the implants for each bird used in this study. It is therefore possible that the secretion of CORT from the implants was for a shorter period and less extensive than predicted from the validations we performed on birds held in smaller cages. Moreover, there is still uncertainty in the role of metyrapone as an inhibitor of CORT synthesis (Freeman and Flack 1984). For avian species, some studies found metyrapone to greatly decrease CORT levels (e.g. Nagra et al. 1965, Frankel et al. 1967, Culbert and Wells 1975, Zachariasen and Newcomer 1975) while others found no evidence that metyrapone constrained CORT secretion (Buckland and Blagrave 1973, Wilson and Cunningham 1980).

The differences between CORT$_f$ in the CORT and metyrapone treatments to CORT$_f$ in the sham treatment were significant only after correcting for pre-treatment CORT$_f$ (i.e. CORT$_f$ of feathers plucked prior to experimental manipulation), sex and site of origin. This is the first study to reveal persistence among individuals in CORT$_f$ across different moult periods, albeit one being natural and the other being experimental. By accounting for these effects in our models, we have shown that males with higher pre-treatment CORT$_f$ had higher levels of CORT$_f$ in feathers regrown after experimental treatment, and the response in CORT$_f$ to each treatment was stronger in individuals from Torquay. This outcome therefore emphasises the importance of acknowledging and accounting for inter-individual differences in propensity to express particular CORT$_f$ levels when designing field-based studies to estimate population-level differences in stress levels from feather analyses.

Our analysis of treatment effect on fault bars suggests the implants did compromise feather quality in a manner expected for elevated plasma CORT (Strochlic and Romero 2008). We found not only a significantly higher average fault bar score in feathers which grew under induced moult in captivity, but also higher average fault bar scores in feathers of birds treated with CORT implants. The greater occurrence of fault bars in sham-treated birds than in the initially plucked feathers that were moulted naturally may reflect the relatively high plasma CORT for sparrows in the sham-treated birds. As found in our validation experiment, sparrows with sham implants had higher levels in comparison to their free-living counterparts in Torquay, where levels were found to be 8.1 ng ml$^{-1}$ during the pre-moulting period (W.A Buttemer, unpubl. data). Occurrence of fault bars on feathers of birds treated with sham implants may also be a consequence of inducing moult by plucking, as induced moult has
been shown to negatively affect feather mass (Dawson 2004) and length (Hall and Fransson 2000).

CORT\textsubscript{f} did not explain fault bar scores on feathers under the different treatments. Fault bars are considered to reflect acute stress (Jovani and Diaz-Real 2012) that results from a disruption in protein allocation at that specific time, as opposed to CORT\textsubscript{f} which reflects a summary of CORT secretion during the entire period that a particular feather is synthesized. The lack of correlation between fault bars and CORT\textsubscript{f}, as well as the lack of differences in the location of fault bars on the feather despite measured temporal differences in plasma CORT levels is sparrows in the validation study suggests that there may be tremendous individual variation in how a given amount of plasma CORT affects feather synthesis (e.g., nutritional differences, protein turnover rate differences, etc.).

We did find a higher occurrence of fault bars on tail feathers than on wing feathers. As suggested for other species (e.g. King and Murphy 1984, Hawfield 1986, Bortolotti et al. 2002), the trend of restricting fault bars mainly to tail feathers (the 'fault bar allocation hypothesis'; Jovani and Blas 2004) is a result of natural selection favouring allocation of more resources to feathers that are most critical for high-powered flight to minimize impact on flight performance at the expense of those less important in this regard (Sarasola and Jovani 2006).

In conclusion, our research has validated the use of feathers as a measure of CORT levels in wild birds. Our results further support those of Lattin et al. (2011) and Fairhurst et al. (2013) in showing a correspondence between plasma and feather CORT. However, we also demonstrated that caution must be taken when using non-invasive methods to infer plasma levels of GCs. Most importantly, we have shown that variance in CORT\textsubscript{f} among individuals was significantly correlated with historic differences in CORT\textsubscript{f} prior to subsequent feather replacement events, irrespective of treatment. This has important ramifications for those attempting to infer population-level differences in stress based on CORT\textsubscript{f} evaluations. We also found that fault bars on most feathers were at similar locations irrespective of the expected temporal differences in plasma CORT among treatments. This challenges the assumption that CORT\textsubscript{f} found in different parts of the feather may be used as a time line in relation to plasma CORT. While our results confirm and encourage the use of CORT\textsubscript{f} as one of the measurements of stress, we nevertheless suggest caution is needed when inferring plasma hormone levels using this non-invasive method.
Acknowledgement

We thank Gabby Hales for her help in the lab in preparing and analysing the samples.
References


Chapter 7

General Discussion

Yaara Aharon-Rotman
When planning the conservation of species we should identify the key areas and moments in
time with the greatest importance for population maintenance. To identify these instances in
space and time for migratory shorebirds from the east Asian-Australasian flyway (EAAF) I
used a holistic approach to include the main factors that have been suggested to constrain
these migrants along their migratory chain during each of the stages in the annual cycle.

Breeding grounds

An obvious starting point for such a holistic analysis is the breeding grounds. It is after all
here where most long-distance migratory shorebirds that fly up and down the EAAF see their
first light. Fluctuation in breeding success of high-Arctic nesting shorebirds is mainly
associated with the prevailing summer temperatures on the breeding grounds (chapter 2).
This association is probably mainly driven by the close relation between insect abundance
and daily temperatures in the Arctic (Tulp and Schekkerman 2008). A shift in the timing of
peak food abundance due to a climate-change induced increase in temperatures may cause a
mismatch between chick growth period and their essential food. Over the past few decades,
some species have indeed been found to shift their breeding timing, possibly adaptively, as an
adjustment to peak food abundance (e.g. Høye et al. 2007, McKinnon et al. 2012). However,
arriving on the peak food abundance period is not the only important aspect in timing of
arrival at the breeding grounds. Snow cover, competition and predation may change as well
as a result of climatic change and it is therefore yet unclear if these adjustments to food peak
period are fully compensating for any loss in fitness as a result of climate change (Lindström
et al. 2014).

A possible future challenge to Arctic nesting birds also relating to the warming temperatures
is the extended range of competing and predatory species that were thus far limited to lower
latitudes. For example, red fox *Vulpes vulpes* is becoming more abundant in northern Canada,
replacing the arctic fox *Alopex lagopus* due to changes in food availability as a result of
climate change (Hersteinsson and Macdonald 1992). Predation pressure may thus increase, as
birds will have to cope with new predators. Humans may also extend their activities
northward as temperatures increase. Besides the direct disturbance by humans visiting sites
where shorebirds are nesting (Morse et al 2006), increasing such activities as tourism and
mining will escalate pollution and disturbance to high latitude nesting birds. In high-Arctic
Alaska for example, oil and gas development, mainly on coastal zones, are threatening
shorebirds habitats (Alaska shorebirds group). In addition, humans will make effort to control insects, which may use as food for chicks (Rehfisch and Crick 2003).

Lemming population cycles have also been suggested to indirectly generate cycles in breeding success of shorebirds and geese through a trophic cascade (Martin and Baird 1988, Summers et al. 1998, Bêty et al. 2001, Blomqvist et al. 2002). However, lemming cycles have changed and even disappeared in parts of the Arctic (Kausrud et al. 2008, Gilg et al. 2009, Menyushina et al. 2012). These changes may also have affected the breeding success of shorebirds and geese, as these have too disappeared in the last 30 years (chapter 2). Currently, these changed conditions have not (yet) resulted in any marked changes in trends in breeding success across years. Declining numbers of shorebirds along the EAAF are therefore more likely related to changing conditions at stop-over and the main non-breeding (‘wintering’) sites.

*Why are lemming cycles fading?*

If changing cycles occurred in lemming populations only, the natural tendency of populations to drift in and out of cyclicity could serve as an explanation. But increasing evidence of similar changing population cycles in other Arctic animal species (e.g. voles, snowshoe hares and forest Lepidoptera) support the involvement of a common, large-scale effect, such as climate change (Ims et al. 2008). In the case of lemmings it has been suggested that climate warming has increased the icing and hardening of the snow surface, reducing food availability for lemmings (Kausrud et al. 2008). Nonetheless, evidence of returning vole cycles in south Finland in the early 2000s after being absent for five years may refute this theory (Brommer et al. 2010) or may simply be a brief local reversal of a long term trend.

As an alternative to the climate change hypothesis, we here suggest that changing predation pressure due to increasing numbers of Arctic breeding geese might also explain changes in lemming cycles. Over the last 50 years the numbers of Arctic-breeding geese wintering in Europe and North America (but not in north-east Asia) have increased dramatically as a result of agricultural intensification and reducing winter mortality (Boyd 1987, Fox et al. 2005). The resulting increase in breeding geese across large parts of the Arctic may have increased prey availability for local predators, potentially stabilizing their numbers at a relatively high level, even during periods of low lemming abundance. In turn this dampened periodicity in predator numbers in the breeding areas of the geese may have reduced cyclicity in lemming
populations.

**Wintering (main non-breeding ground)**

Although good conditions during the early stages of life at the breeding grounds are obviously crucial, conditions on the other hemisphere on the wintering grounds, 10-14 thousand kilometres flight down the EAAF, may be just as decisive for a successful and productive life. Using physiological tools and the ruddy turnstone as a model species, we assessed the quality of three different wintering sites: King Island (Tasmania), east South Australia and Broome (north Western Australia).

Physiology plays an important role in improving our knowledge of how habitat quality affects individual fitness and therefore, survival and reproductive success (Wikelski and Cooke 2006). Our analyses support that birds wintering on King Island and South Australia have better health measurements than birds wintering in Broome, and yielded a possible explanation for the advantage of flying an extra distance to winter on the southern side of Australia, rather than wintering in north Western Australia (Chapter 5). For those birds opting for southern Australia, the higher costs of thermoregulation and flight are compensated by better feeding conditions (see also Chapter 3) and lower blood parasite infection rates (though not significant in our study).

Birds wintering in favourable locations have better survival and breeding success. The poorer health condition in the Broome population, in comparison to birds from South Australia and King Island, may reinforce their site fidelity by limiting their ability to explore other opportunities for better feeding sites. On the other hand, as feeding conditions are poor we may expect a shift in wintering site, as birds will desperately look for better possibilities or be selected against. Moreover, it is not yet known whether juveniles winter at the same location following their parents. As juvenile Arctic shorebirds migrate independently from adults, there must be a strong genetic basis for their migratory behaviour (Lindström et al. 2014). If indeed juveniles are following their parents to the wintering ground, it implies that the population in Broome would keep deteriorating and may include only the weak individuals. All this remains speculative until a comparative analysis on the fitness of birds wintering in Broome and South Australia could be conducted. That will, however, be a long shot, given that it will be very hard to evaluate, for instance, the life-time reproductive success of individual shorebirds. However, given many years of banding data for both areas, a
comparative analysis of local-survival and site-fidelity might be feasible and very worthwhile. Regarding reproductive success, differential juvenile percentages are another indicator of fitness. Thus far, these, however, have not shown any differences between the two wintering locations.

Measurements of stress biomarkers, fuelling rates and parasite infection in different wintering habitats highlight the importance of combining ecological and physiological tools when assessing habitat quality. However, some physiological measurements, mainly non-invasive tools, should be used with caution. Our validation study for the use of corticosterone (CORT) from feathers as a measure of long-term elevated levels of plasma CORT (Chapter 6), confirms the validity of this technique in house sparrows *Passer domesticus*. However, at the same time highlights inter-individual variations and the importance of accounting for biological and environmental factors (e.g. sex, site of origin and baseline CORT) before inferring a broad application of the technique to other species and environments.

*Migration-strategies*

Fuelling rates, i.e. the rate at which body stores are being accumulated, are of critical importance to migrants, determining the time it will take them to complete their migratory journey and their risk of starvation during the arduous trip. Typically, this fuelling rate appears to increase with latitude (Chapter 3). This latitudinal cline in fuelling rate has far-reaching consequences for how migrants go about planning their migratory journey. As outlined in chapter 3 we predict, and confirm, that this latitudinal trend in fuel deposition rates makes migrants fly non-stop from sites at high latitude to a site at the same latitude or higher. The latitudinal trend in fuelling rates explains the observed migration strategies in many cross-equatorial migrants, congruent with predictions from the time-minimisation hypothesis.

The largest disturbances and threats migrants are facing are observed in the staging sites within the Yellow Sea area, located at a latitude of approximately 38°N. As migratory birds may disproportionately rely on high latitude staging areas, these deteriorating sites could act as the primary ecological bottlenecks for the migratory shorebirds that have relied on them in the past. Although most of the studies along the flyway found the Yellow Sea area as the weakest link where conditions are deteriorating at a rapid pace, the latitudinal patterns in fuelling rates also emphasise the importance of maintaining the quality of major wintering sites in Australia. These sites are paramount for migrants to fuel up and reach the energy
demands prior to embarking on long flights when crossing the equator. Thus, the concept that migratory birds may disproportionately rely on high latitude staging areas needs to be considered when prioritising conservation efforts.

**Migration- deteriorating stop-over sites**

Using the ruddy turnstone as an example, the importance of the wintering site in Australia was further underlined when we tested the implications of habitat loss and degradation in sites along the EAAF (Chapter 4). Even a small decrease in the intake rates at the South Australian site for birds preparing for migration had severe consequences on reproductive success and survival, because birds failed to fuel sufficiently to (timely) cover the flight distance. Given the strategy used by some migratory shorebirds to fly long distance from sites at high latitudes to skip low quality sites around the tropics, we confirmed that conserving high quality wintering sites in Australia is crucial for maintaining the population.

Equally important, our modelling results further confirmed that changing conditions along the EAAF are also threatening the future of many migratory shorebirds populations. Specifically a reduction in intake rates in Taiwan, Sakhalin and the Yellow Sea area, three important staging sites at high latitudes, were found to affect breeding success. As mentioned earlier, extensive reclamation of intertidal areas in the Yellow Sea (MacKinnon et al. 2012, Murray et al. 2014), as a result of the “new great wall” (Ma et al. 2014), is one such example that may impair the reproductive success of migrants, therefore contributing to declining numbers.

The next big step forward would now be to challenge our model and test its applicability in real life. As one approach we could infer an individual’s ability to respond to environmental fluctuations by analysing and comparing multiple tracks across years. Individuals that repeat the exact route for multiple years and are thus highly “conservative” may indicate to lack the ability to make adjustments to changing environmental conditions. A more challenging but definitely more informative challenge of migration models may be achieved by providing unlimited or restricted feeding opportunities to birds in the field. These manipulations are expected to drive an adjustment of departure date or flight distance, which can be recorded with tracking devices. Such an experiment is indeed challenging to apply (based on trials done in earlier stages of this thesis), but will provide valuable data to verify the validity of our model (Chapter 4) and similar models developed for shorebirds (Bauer et al. 2010).

**Challenges in the conservation of long distance migratory shorebirds**
The last two centuries have been marked by declines in migratory populations and losses of migratory behaviour (Wilcove and Wikelski 2008). In conjunction with the wider migratory research field, the findings presented in this thesis indicate that the challenges faced by migratory animals can be primarily attributed to habitat loss. Coastal habitats, which are favoured by shorebirds, are increasingly being modified and disturbed by human activity. These threats are greater for long distance migratory birds that use multiple sites on their annual route, which may all be important for their survival and reproduction. Moussus et al. (2011) found that the species that are able to adjust to climatic changes and are able to make rapid adjustments in breeding phenology were the short distance migrants with the broadest ecological niches (“generalists”). We could therefore assume that on the other side of the scale are habitat specialists, long distance migratory species.

For efficient prioritisation in conservation plans for mobile species, there is a need for action that takes into account the dependencies among sites, which are dynamic in space and time (Runge et al. 2014). The challenge is even greater when these multiple sites cross jurisdictions. There are, however, many international treaties that aim to tackle the problem at a regional or species-specific level (Global Convention on Migratory Species; http://www.cms.int/en/legalinstrument/cms).

Although migratory species are thought to be exposed to more threats than sedentary species (Newton 2004, Milner-Gulland et al. 2011), their ability to move between sites might also be advantageous, potentially allowing them to move away from deteriorating conditions. However, as migratory shorebirds are long-lived species with strong genetic basis for migration behaviour, they may lack sufficient capacity to evolve at a rate matching the rates of rapid global environmental changes, as any change in behaviour should happen through natural selection and microevolution. Therefore, in order not to succumb and disappear from the face of the earth, they must show adaptive flexibility to cope with these changes (Lindström et al. 2014).

Indeed, many migrants show some degree of adjustment in response to global changes, while the observed reaction of others is at a slower pace or completely absent (e.g. Moussus et al. 2011). Failing to adjust to environmental changes, e.g. mismatch between birds and their preferred prey, may have detrimental consequences for bird populations (Lindström et al. 2013).

**Future directions**
Future research should be carried out to further demonstrate the detrimental impact various factors may have on migratory species. Decision makers require firm evidence before they are sufficiently convinced to make decisions that are not based on economic grounds exclusively and also take the environment into consideration. Therefore, while causal uncertainty remains on the impact of global change processes, more precise data needs to be gathered on shorebirds and their habitats along the flyway. A valuable first step in the conservation process would be to evaluate the habitats that form the system of protected areas (Colwell 2010). The EAAF, where global changes are taking place at an unprecedented pace, has an immense shortage of knowledge about the carrying capacity of stop-over habitats and the locations of where reclamation projects are happening (Leyrer et al, in prep). There is also a need for more empirical data on how shorebirds cope with the loss of their preferred staging sites.

Improving our knowledge on habitat quality could be done using conservation physiology tools (Wikelski and Cooke 2006), such as the ones included in this thesis. These tools not only allow assessment of the quality of habitats, but also the physiological effects on populations of variation in quality. For example, measuring the amount of food available to shorebirds in one staging site may overlook other factors such as predation disturbance and energy expenditure which reduces the intake rates for the animal. By combining habitat characteristics with physiological parameters, e.g. fitness or stress levels, we could make conclusions based on the net effect on the animal.

There is a large amount of evidence on how pollutants affect humans and wildlife worldwide and an increasing attention to pollutants that persist in the environment (Jones and De Voogt 1999, Herceg-Romanic et al. 2014). There is a need for more information on the presence of pollutants in shorebird areas along the EAAF. Although levels of DDT, PCBs and PBDEs were found to be low in limited number of liver samples from three different shorebird species from the EAAF (Box. 1), levels of some pollutants in some areas along the EAAF (mainly on the south east Asian coast) were found to be high in sediments (Ma et al. 2001, Zhang et al. 2009). Further to this, the effect of pollutants on migratory animal’s behaviour and survival needs to be evaluated. Most of the research this far has focused on sedentary species. Thus, more studies on migratory species are needed to verify the hypotheses and assess the direct impact of toxicants on migrants (Klaassen et al. 2012).
It should be noted that the conservation of coastal wetlands is not only important for maintaining shorebird populations. Wetlands play a number of important ecological roles (e.g. absorbing pollution, providing spawning and nursing grounds for many aquatic organisms and providing ecological barriers against extreme weather events) (Ma et al. 2014). Accordingly, they should be of high priority in conservation efforts.

By identifying the challenges to migratory shorebirds in key sites along the flyway, we should be able to protect and maintain a sufficient network of suitable staging sites to ensure maintaining populations of these shorebird species.
References


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Box.1: Levels of Persistent Organic Pollutants (POPs) in Migratory shorebirds of the East Asian-Australasian Flyway

Introduction

A range of global change processes including habitat loss and deterioration have been considered as the main factors explaining declining numbers of wild animals worldwide. Deteriorating habitat may also include increased exposure to environmental pollutants such as organochlorines and trace elements (Hua et al. 2015, Morrison et al. 1994).

Persistent Organic Pollutants (POPs) including DDT, PCBs and PBDEs (see Methods for background information on these compounds have received much attention due to their persistent nature, bioaccumulation, long-range environmental transport and toxicity to wildlife and humans (Jones and De Voogt 1999, Herceg-Romanic et al. 2014). Their presence in sediments, even if at low levels only, is therefore a call for attention (Li et al. 2014), their tendency for bioaccumulation being supported by higher levels of contaminants in organisms rather than in sediments (Li et al. 2014), and in fish rather than in invertebrates, stemming from lower trophic levels (Tian et al. 2010).

The increasing reliance of wild animals on anthropogenic habitats put animals at greater risk for exposure to pollutants. This holds particularly true for migratory animals due to their characteristic behaviour and physiology. Migrants rely on the deposition of fuel stores to allow their long-distance migrations. During periods of migratory fuelling their food intake vastly exceeds their daily energy needs, potentially exposing them to increasing amounts of toxicants (Debier et al. 2006, Klaassen et al. 2012). Moreover, fuel stores for mostly contain fat and lipid-soluble organic pollutants are therefore likely to accumulate in these stores (Harris and Elliott 2011) to be released during endurance flight as fat is being metabolized.

Shorebirds are particularly vulnerable to exposure to environmental pollutants due to their diet of aquatic and marine invertebrates from sediments in estuaries and agricultural fields, where pollutant concentrations are often elevated (Braune and Noble 2009). There has been little experimental work on the toxic effects of POPs in birds (Stickel and Stickel 1969, Van Velzen and Kreitzer 1975), but suggestive effects of POPs on bird mortality (Wurster et al. 1965, Blus et al. 1985) and reproductive success (Hoffman et al. 1996) have been recorded.
The East Asian-Australasian Flyway (EAAF), a migratory corridor of importance to about 8 million shorebirds, has experienced declines in biodiversity, loss of ecological services, and an increase in ecological disasters (MacKinnon et al. 2012). As a result, numbers of some migratory species along the route are declining at rates of up to 5% per annum (R. Fuller, pers.comm). Traces of POPs have been found at a range of sites along the EAAF regularly frequented by shorebirds, including their breeding, migratory staging and wintering grounds.

**Staging sites**

Much of the studies on POPs along the EAAF have been conducted in China, and more specifically, in the area around the Yellow Sea. The Yellow Sea, bordered by China and North and South Korea, is one of the most important stop-over sites for migrating shorebirds along the EAAF. It is also one of the most developed zones in China and South Korea and has recently been the subject of a number of studies on levels and effects of POPs in the environment. Because the water exchange rate is very low in this area, there is a high pollutant burden on its sediments. As a result Bohai Bay, located in north Yellow Sea, is being considered one of the most polluted sea areas in China (Zhang et al. 2009).

Although its use was banned 20 years ago, high levels of DDT were found in sediments in some sites in the Yellow Sea area, with the highest levels detected near Qingdao (Ma et al. 2001, Hu et al. 2009, Zhang et al. 2009), as well as in all 13 species of seafood, including species of fish, crustaceans and molluscs collected on the coast of Zhoushan along the East China Sea, south of the Yellow Sea (Wang et al. 2014).

PCBs were identified in many intertidal sediments within the Yellow Sea area. However, the levels were lower than reported in some other estuaries in the world, e.g. Gulf of Main, Tiber estuary, Baltic Sea (Ma et al. 2001). On the other hand, concentrations of PBDEs and PCBs in fish from the Yellow and the East China Sea were high compared to other regions studied in China and some areas in the USA (Liu et al. 2011).

In Taiwan, a major stopover site for some migrants along the EAAF, levels of PCBs in the air are lower relative to other Asian countries, including China and Japan. Periods with increased levels of PCBs in the air were found to involve Asian Dust Storm events, where dust particles from the desert of central Eurasia are carried east by prevailing winds and deposited in east Asia and southern China (Chi et al. 2008).
In Indonesia, PCBs and DDT are the main contributions to the total POPs found in fish, but levels are still lower than levels found in Japan. Importantly, when the same areas were sampled in 2003, five years after the first sampling event, lower values of PCBs and DDT were detected, indicating decreasing levels in the environment (Sudaryanto et al. 2007).

*The Arctic breeding ground*

Transported by winds and ocean currents, many toxins originating from more southern latitudes accumulate in the Arctic (Law and Stohl 2007). As a consequence of bioaccumulation, many POPs such as PCBs, DDT and PBDEs are found in Arctic biota and humans, with birds and mammals at the top of the marine food web being at higher risk of exposure to high levels of POPs (Letcher et al. 2010, Erikstad et al. 2013). Most data on levels of contamination is from the Canadian Arctic, Greenland and Norway, where examination of PCBs and DDT in different animal groups found highest levels in polar bears from the Hudson Bay, east Greenland and Svalbard (e.g. Letcher et al. 2010). Data on levels of POPs in the Russian Arctic are lacking (or at least unavailable in English). In two rivers in east Siberia, part of the areas where shorebirds from the EAAF breed, levels of DDTs in sediments were found to be low, with most values below the 5ng l⁻¹ detection limit, and decreasing over the period 1988-1994 in water, sediments and fish, probably as the use of these pesticides has been curtailed starting 1970 (Muir et al. 1997, Zhulidov et al. 2002). These DDT levels thus suggest limited risk to humans and wildlife in this part of the Arctic.

*Australian Wintering grounds*

The use of DDT is banned in Australia since 1987, but can still be detected mainly in the soil of contaminated cattle tick dipesites as well as disposal sites of industrial waste in New South Wales (Bell 2009). As shorebirds wintering in Australia are not expected to visit these areas, their exposure to DDT is probably low in this period of their annual cycle. Australia’s PCBs management plan is aimed at destroying all PCBs containing materials with concentrations of > 50 mg/kg by 2009. So far large quantities have been removed and Australia’s national implementation plan expects to have banned all use of PCBs by 2025 (Australian Government 2006).

PCBs and PBDEs levels are low in Australian wildlife and humans. A study on a large set of chemical compounds, including PCBs, in breast milk found levels to be lower compared to international standards (Australian Government 2006). Although levels of PCBs in certain
seafood in Moreton Bay were elevated relative to other marine/estuarine and retail fish from other areas in Australia, they were still below the EU maximum limit for contaminants in food manufacturing and agriculture (Paepke et al. 2008). Also examination of POPs in fish from fish markets in Adelaide revealed low levels of PBDEs in local products compared to concentrations in fish from Thailand and Vietnam (Shanmuganathan et al. 2011). Although comparisons between studies is often difficult as different laboratories analyse different suites of PBDEs congeners, where comparison was possible, PBDEs levels in marine biota in Australia (mainly turtles and dugongs) were low relatively to reports from marine biota in the northern hemisphere (Asia, Europe, Canada and the USA) (see review of studies on marine biota in Hermanussen et al. (2008)).

The above reviewed presence of POPs in sediments, prey and higher vertebrates along the EAAF, identify a few hotspots for potential contamination by pollutants and notably so on migratory staging sites along the SE Asian coast. We thus examined levels of PBDEs, DDT and PCBs in the liver of three species from the EAAF that have contrasting breeding ranges and migratory routes and are also expected to be operating at different trophic levels and may therefore show differential contamination levels: Red-necked Stint *Calidris ruficollis* (RNS), Greater Sand Plover *Charadrius leschenaultii* (GSP) and Great knot *Calidris tenuirostris* (GK).

While GSP is presumed to breed in central Asia (north-west China, Mongolia and southern Siberia at about 40-60°N), GK breed in the low Arctic (60-70°N) and RNS breed in the high Arctic (60-80°N) (Minton et al. 2011). The migratory route of the GSP is more westerly than the other two shorebird species visiting Australia (Minton et al. 2011). While GK and RNS are dependent on stop-over sites along the Yellow Sea coast, GSP has been recorded both on passage in eastern Asia (Barter 2002, Minton et al. 2011), with most of the flag-sightings from Taiwan, Hong-Kong and Vietnam (Minton et al. 2011). GK and GSP are larger than RNS and are thus likely to ingest prey from a higher trophic level than RNS and are thus more prone to bioaccumulation. Moreover, GK mainly forage on bivalves, GSP on crabs, while RNS on worms, snails, and biofilms. Different macrobenthos might expose them to different levels of POPs.

Given the large number of evidence of high levels of POPs along the Asian coast, mainly in the area of the Yellow Sea, the expectation is thus that GK and RNS, which are dependent on these sites to fuel on the way to the breeding grounds, will have higher content of pollutants.
in liver rather than GSP. On the RNS is possibly operating at a lower trophic level and may thus be less contaminated than GK and GSP.

**Methods**

*Summary information on POPs*

The organochlorine compound Dichlorodiphenyltrichloroethane (DDT) was first used intensively to control malaria and typhus during World War II, followed by an extensive use in agriculture (Hayes 1991). Not long after, concerns were raised on the negative effects of DDT to health of humans and wildlife (Cottam and Higgins 1946, Carson 1962). Although DDT was an effective tool in pest control, the negative environmental effects resulted in DDT being banned in many countries starting in the early 1970s. However, as it is a cheap and effective anti-malaria pesticide, it is still being used in many tropical countries in malaria control (Alava et al. 2011). Due to its persistence in the environment, its continued use and ability to be transported atmospherically, its metabolites (mainly DDE and DDD) continue to accumulate worldwide (Lubick 2007).

DDT and its metabolites in wildlife have been related to both increased mortality and a range of sub-lethal effects (Blus 2011), including eggshell thinning (Ratcliffe 1967, Hickey and Anderson 1968) and reproductive success (Heath et al. 1969). Environmental exposure to DDT was also found to alter brain function in birds (Iwaniuk et al. 2006).

Polychlorinated biphenyls (PCBs), an industrial chemical used in many products including as additive to paint and carbonless copy paper (Australian Government 2006), were manufactured from the 1930s to 1980s-1990s, but due to their bioaccumulation and toxicity in wildlife worldwide (Harris and Elliott 2011) they are globally banned since 2004 in accordance with the Stockholm Convention on POPs. As a result, since the 1990s, monitoring programs have reported a decline in tissue burdens of PCBs in wild birds (Norstrom and Hebert 2006). There is, however, still some evidence on their current use in China (Hu et al. 2009, Zhang et al. 2009, Wang et al. 2014). PCBs are lipophilic and as such tend to accumulate in fatty tissues of organisms. Most of the studies on the potential physiological effects of PCBs have focused on mechanisms related to reproductive success and found negative effects on parental behaviour, clutch size, development, egg quality and hatching success (see review by Harris and Elliott (2011)). In addition, exposure to PCBs has been associated with decreased survival rates (e.g. Elliott et al. 1996, Breton et al. 2008) and endocrine disruption (Beineke et al. 2005, Das et al. 2006).
Polybrominated diphenyl ethers (PBDEs) have a similar chemical structure to PCBs (but have chlorines replaced with bromines and an oxygen bonding the two rings), and are used in flame retardants since the 1980s. Early evidence of mammalian toxicity led to restrictions on the manufacturing of these products (Harris and Elliott 2011). Although there is only limited evidence for negative effects of BPDEs on wild birds, PBDEs have been suggested to negatively affect reproduction and physiology in a way similar to PCBs (Beineke et al. 2005, Das et al. 2006, Harris and Elliott 2011).

**Sample collection**

During shorebird catching by the Australian Wader Study Group in north Western Australia (NWA), Victoria and South Australia in the years 2010-2013, occasional bird casualties were collected. We dissected individuals of the species RNS (10 from NWA, 5 from South Australia and 2 from Victoria), GSP (12 individuals from NWA) and GK (12 individuals from NWA) and extracted their liver.

Samples were pooled per species with three replicates per species. Samples were then sent to the National Research Centre for Environmental Toxicology (Entox), The University of Queensland, for analysis of POPs profiles.

**Results and Discussion**

The main pollutant found in our species was ppDDE (DDE), which is the main breakdown product of DDT. Highest levels were found in GK, followed by GSP and RNS (Fig.1). In comparison with other studies, levels found in our study were low. In Canadian shorebirds for example, Braune and Noble (2009) found highest DDE levels in Lesser Yellowlegs *Tringa flavipes* (29.2 μg/g lipid weight) and Whimbrel *Numenius phaeopus* (5.77 μg/g lw). The lowest levels were found in long-billed Dowitcher *Limnodromus scolopceus* (non-detectable) and in Hudsonian Godwit *Limosa haemastica* (0.044-0.9 μg/g lw), which are comparable to the highest levels found in our study in GK (0.044 μg/g lw).

PBDEs concentrations in our study species also show low levels of exposure in GK and GSP, and below detectable levels in RNS. The family of PBDEs consist of 209 congeners (from which we examined 18), typically PBDE-47 would dominate, and is found in many wild animals (e.g. Liu et al. 2010). Interestingly, this is not the case for GK, where only low levels of PBDE-49 and PBDE-100 were found. PBDE-49 is less studied, and was recently
found to have a negative effect on survival, tail curvatures, and stress in Zebrafish with doses exceeding 2000 μg/l (McClain et al. 2012). We did not find any traces of PCBs in our samples (Fig.1).

POPs tend to bio-accumulate and as a result levels are higher in higher trophic levels. In our three study species, GK and GSP are bigger (115-248 g and 55-121 g, respectively) than RNS, which is one of the smallest long-distance migratory shorebirds found in Australia (18-51 g). GK and GSP may therefore forage on average at a higher trophic level since they devour larger prey than RNS. Furthermore, RNS has been suggested, along with other (small) sandpiper species, to forage intensively on biofilm (low trophic level) (Kuwae et al. 2012). These feeding limitations may explain the relatively high levels of DDT and PBDEs found in GK compared to RNS. Levels found in GK were also higher than levels found in GSP. These differences may be explained by the fact that GK makes more extensive use of coastal, highly contaminated areas than GSP, the latter taking a more westerly route away from Eastern China Sea and Yellow Sea.

Most of the birds from which samples were analysed died long after their arrival on the wintering grounds. The low levels of POPs found in the livers of our study species are likely a resultant of low exposure to contaminants from food consumed on the wintering ground (Australia) and either low exposure to contaminants further up the EAAF or a washout of contaminants ingested earlier. This washout may have been further amplified by the fact that most of the birds in our study were collected during the period of pre-migratory preparation, when birds are fuelling up extensively before embarking on their long journey to the northern hemisphere breeding grounds. Thus, we cannot rule out that the low levels detected may be remnants of exposure to higher levels of contamination from other stopover sites along the flyway, but were diluted by food consumed in Australia.

Although levels found in our samples are relatively low, negative effects on the animals may still arise if they are under heat stress. Most of our samples were from animals collected in March in Broome, where temperatures are high (25.4-33.9°C on average) and may lead to temperature-dependant toxicity, as shown for herbivore mammals (Dearing 2013). In hot environments, where ambient temperatures exceed the animals’ upper critical temperatures (UCT), overall liver metabolism is reduced, limiting the ability of the animal to digest toxicants (Dearing 2013). Given the low levels of toxicants detected in our species, this
mechanism of increased toxicity during heat stress may not have applied.

**Figure 5**: levels of ppDDE (the main breakdown product after exposure to DDT; left panel) and three congeners of PBDE (47, 49 and 100; right panel) as detected in liver samples of three migratory shorebird species: Red-necked Stint (RNS), Greater Sand Plover (GSP) and Great Knot (GK), from samples pooled by species (three replicates per species). Black bars represent standard error. Levels of PCBs were below the detection limit and were therefore not presented.
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