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**SEASONAL VARIATION IN MIGRATORY BODY
CONDITION, CORTICOSTERONE SECRETION,
OXIDATIVE STRESS AND BIOTRANSFORMATION
ACTIVITY IN BARN SWALLOWS
(*HIRUNDO RUSTICA* L.)**

by

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ABSTRACT

UNIVERSITY OF TURKU

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RAJA-AHO, SARI: Seasonal variation in migratory body condition, corticosterone secretion, oxidative stress and biotransformation activity in barn swallows (*Hirundo rustica* L.)

Doctoral thesis, 103 p.

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I studied the associations between migration-related physiological regulation (corticosterone) and body condition of barn swallows (*Hirundo rustica* L.). An additional purpose was to determine whether oxidative stress and biotransformation activity vary seasonally. Since physiological regulation, biotransformation activity and the stress involved may be important factors for body condition during migration; they may have direct effects on migration success. This in turn may influence other important life history stages, such as breeding and moult. In the thesis I used barn swallow data of the Finnish Ringing Centre (1997–2009), consisting of all juveniles ringed in the nests and recaptured from night roosts later the same autumn. Before the autumn migration in Finland I also captured, ringed and sampled barn swallows from night roosts in 2003, 2006, 2007 and 2011. Samples preceding spring migration in South Africa were collected in 2007.

Juvenile barn swallows started to migrate southward in mid-August (first broods). Second broods started their migration at a younger age and almost a month later than first broods (mid-September). Barn swallows increased body mass and accumulated fat for the autumn migration. In the course of the autumn they seemed to be able to prevent the loss of energy already accumulated, since the proportional overnight mass loss, fat loss and faecal production decreased. Surprisingly, corticosterone, the major energy-regulating hormone in birds, seemed not to be involved in the fuelling process. Previous studies with warblers, sparrows and shorebirds had shown that during migration, the baseline levels of corticosterone were elevated in order to facilitate fuelling. It is possible that for Finnish barn swallows the most important fuelling place is in southern Europe, since northern and eastern populations migrate via the Balkan Peninsula. However, the adrenocortical stress response of Finnish barn swallows in good body condition was lower than that of those in poor body condition. Birds clearly suppressed the response, probably to prevent the catabolic effects of excessive corticosterone levels; birds cannot afford to lose muscle mass before migration.

South African barn swallows had high levels of baseline corticosterone, but this may have been associated with the high oxidative damage and biotransformation activity of those birds. Barn swallows in spring and summer had low biotransformation activity and intermediate oxidative stress, which was probably related to breeding. Autumn birds had low biotransformation activity and oxidative stress but high redox enzyme activities in some migration-related enzymes.

Key words: brood, energy accumulation, fat score, body mass, migration physiology, roost, corticosterone, oxidative stress, biotransformation activity

TIIVISTELMÄ

TURUN YLIOPISTO

Biologian laitos/Matemaattis-luonnontieteellinen tiedekunta

RAJA-AHO, SARI: Haarapääskyjen (*Hirundo rustica* L.) muuttokunnon, kortikosteronierityksen, oksidatiivisen stressin ja biotransformaatioaktiivisuuden kausittainen vaihtelu

Väitöskirja, 103 s.

Ekologian osasto

Toukokuu 2014

Tutkin työssäni haarapääskyjen (*Hirundo rustica* L.) muuttoon liittyvän fysiologisen säätelyn (kortikosteroni) ja kehon kunnan välisiä yhteyksiä. Lisäksi selvitin oksidatiivisen stressin ja biotransformaatioaktiivisuuden kausittaista vaihtelua. Koska fysiologinen säätely, biotransformaatioaktiivisuus ja siihen liittyvä stressi voivat muuton aikana olla tärkeitä kehon kuntoon vaikuttavia tekijöitä, niillä voi olla myös suoria vaikutuksia muuton onnistumiseen. Tämä puolestaan vaikuttaa muihin elinkierron vaiheisiin, kuten pesimiseen ja sulkimiseen. Tässä väitöskirjassa käytin Suomen rengastustoimiston haarapääskyaineistoa (1997–2009), joka sisältää kaikki pesiltä rengastetut haarapääskyn poikaset sekä samana syksynä ruovikoista uudelleen kontrolloidut yksilöt. Lisäksi otin kiinni, rengastin ja keräsin näytteitä haarapääskyiltä yöpymisruovikoista Suomessa ennen syysmuuttoa vuosina 2003, 2006, 2007 ja 2011. Ennen kevätmuuttoa vuonna 2007 näytteitä kerättiin myös Etelä-Afrikasta.

Nuoret haarapääskyt lähtivät syysmuutolle elokuun puolessa välissä (ykköspoikueet). Kakospoikueet lähtivät muutolle nuorempina ja vasta kuukautta myöhemmin (syyskuun puolessa välissä) kuin ykköspoikueet. Haarapääskyt lisäsivät painoaan ja keräsivät ihon alaista rasvaa syysmuuttoa varten. Syksyn kuluessa haarapääskyt pystyivät ilmeisesti säästämään jo kerättyä energiaa, sillä yövyttyjen lintujen massan ja rasvan menetys sekä ulosteiden määrä vähenivät. Yllättäen kortikosteroni, joka on lintujen tärkein energiansäätelyhormoni, ei vaikuttanut tankkaukseen. Aiemmat tutkimukset kertusilla, varpusilla ja kahlaajilla ovat osoittaneet, että muuttoaikana kortikosteronin perustaso on koholla, mikä puolestaan lisää lintujen tankkausta. Ilmeisesti haarapääskyjen tärkein tankkauspaikka on kuitenkin Etelä-Euroopassa, koska pohjoiset ja itäiset populaatiot muuttavat Balkanin niemimaan kautta. Toisaalta, Suomessa hyväkuntoisten yksilöiden stressireaktio kiinniottotilanteessa oli pienempi kuin huonokuntoisten yksilöiden. Haarapääskyt selvästi vähensivät stressireaktiotaan, mikä johtunee tarpeesta estää liiallisen kortikosteroni pitoisuuden lihaskudoksia tuhoavaa vaikutusta. Ennen muuttoa linnuilla ei ole varaa menettää lihaskudosta.

Etelä-Afrikan haarapääskyillä kortikosteronin perustasot olivat kohollaan, mikä luultavasti liittyi lintujen korkeaan oksidatiiviseen stressiin ja biotransformaatioaktiivisuuteen. Keväällä ja kesällä kiinniotetuilla linnuilla biotransformaatioaktiivisuus oli alhainen ja mahdollisesti pesintään liittyvän oksidatiivisen vaurion määrä oli keskitasoinen. Syksyn linnuilla biotransformaatioaktiivisuus ja oksidatiivisen stressin määrä oli alhainen, mutta muutaman entsyymin aktiivisuus oli keskitasoinen. Aktiivisuus liittyyneen muuttoon valmistautumiseen.

Avainsanat: poikue, tankkaus, rasvaindeksi, massa, muuton fysiologia, ruovikko, kortikosteroni, oksidatiivinen stressi, biotransformaatioaktiivisuus

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LIST OF ORIGINAL PAPERS

This thesis is based on the following publications and manuscripts referred to in the text by their Roman numerals:

- I Raja-aho S, Eeva T, Suorsa P, Valkama J & Lehikoinen E. Juveniles from second broods set off on autumn migration younger and in better body condition than those of first broods in the barn swallows (*Hirundo rustica* L.). Submitted manuscript.
- II Raja-aho S, Suorsa P, Lehikoinen E & Eeva T. Changes in body mass, fat score and faecal production in juvenile barn swallows (*Hirundo rustica* L.) prior to autumn migration. Manuscript.
- III Raja-aho S, Suorsa P, Vainio M, Nikinmaa M, Lehikoinen E & Eeva T. 2010. Body condition is associated with adrenocortical response in the barn swallow (*Hirundo rustica* L.) during early stages of autumn migration. *Oecologia* 163: 323–333.
- IV Raja-aho S, Lehikoinen E, Suorsa P, Vosoloo D, Vainio M, Nikinmaa M & Eeva T. 2013. Corticosterone secretion patterns prior spring and autumn migration differ in free-living barn swallows (*Hirundo rustica* L.). *Oecologia* 173: 689–697.
- V Raja-aho S*, Kanerva M*, Eeva T, Lehikoinen E, Suorsa P, Gao K, Vosoloo D & Nikinmaa M. 2012. Seasonal variation in the regulation of redox state and some biotransformation enzyme activities in the barn swallow (*Hirundo rustica* L.). *Physical and biochemical zoology* 85: 148–158.
*SR and MK contributed equally to this article

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1. INTRODUCTION

Over the past decades long-distance migratory birds breeding in Europe and wintering in sub-Saharan Africa have experienced considerable population declines compared to more sedentary ones (Berthold et al. 1998; Berthold 2001; Sanderson et al. 2006; Møller et al. 2008). The reasons behind these changes are not quite clear, since evaluating the reasons for any population decline is difficult. Factors influencing migratory populations may operate at their wintering grounds, breeding grounds and/or migration routes (Newton 2004). In general, factors can be predictable (e.g. season, day-night) or unpredictable (e.g. storms, lack of food, predation), short- or long-term, human-induced or natural; all of these are equally stressful to individual birds. Of human-induced factors, at least global climate change, disturbance in breeding and wintering areas, and exposure to various pollutants affect long-distance migratory birds during the annual cycle (Wingfield 2008). In evaluating changes in migratory bird population trends, factors relating to breeding, migration or wintering periods are usually considered independently, while the understanding of seasonal interactions and/or their mutual influence on population trends may be more restricted (Marra et al. 1998; Gill et al. 2001; Gunnarsson et al. 2005). There is a simple reason for this: data on different stages of the annual cycle are hard to obtain.

Migration is not only an energy-demanding life-history stage but also a significant population limiting factor for long-distance migratory passerines (Sillert and Holmes 2002). During their migration birds may travel over large areas on different continents of sometimes hostile terrain, refuel in atypical habitats with many competitors, face adverse weather conditions, suffer from physiological stressors and be exposed to predators, any or all of which may increase annual mortality rates (Newton 2008). Several studies have tried to identify the causes underlying the low over-winter survival rates of sub-Saharan migrants; important factors may include for example habitat destruction, desertification and lower resource availability in the Sahel zone, which in turn can partly be explained by increasingly intensified agriculture (Berthold 2001) and continued aridity since the late 1960s (Nicholson et al. 2000; Foley et al. 2003).

In comparison to sedentary birds, migratory birds need particular adaptations for successful migration, such as the timing of migration, accumulating energy for migration, and finding their way over long distances (Newton 2008). Since successful migration determines the timing and duration of other important stages in the annual cycle, i.e. breeding and moult, it may be an essential factor affecting population trends. Climate

change may cause further losses among some long-distance migratory bird species if they are unable to couple the timing of resource availability with the timing of migration (Schaub et al. 2005; Møller et al. 2008). In order to maximize survival during migration, birds may either try to minimize the time spent in migration or optimize their migration speed (Alerstam and Lindström 1990). The time taken by migration can be reduced by minimizing the time spent on stop-over refuelling and/or maximizing the time in flight (energy reserves). The migration speed, which combines the time spent in stop-over and in flight, may also be affected by the risk of predation and energy costs (La Sorte et al. 2013). Environmental and/or migration related stresses (whether of natural or human origin) are usually associated with changes in body condition. If these changes lead to deteriorated body condition during migration, there may be direct consequences for the fitness of birds.

1.1. Migratory fuelling

For small migrating passerines it is impossible to store enough energy to fly non-stop between distant breeding and wintering areas (Biebach et al. 2000; Schaub and Jenni 2000). Most long-distance migrants perform their migration in several long flights interrupted by stop-over periods. The length of the migration flight determines the size of energy stores needed (Lindström 1991; Kvist et al. 2001; Piersma and Lindström 2002; Rubolini et al. 2002; Wikelski et al. 2003). The migration is speeded up by the ability to accumulate energy efficiently by fuelling at stop-over sites, allowing birds to allocate more time for breeding and/or moulting (Lindström 1991; Pilastro and Magnani 1997; Kvist et al. 2001; Piersma and Lindström 2002).

In small passerines, the amount of subcutaneous fat during migration can increase by up to 50 % of the total body mass (Berthold 2001; Bairlein 2002). Over 90% of energy usage during the migration flight is aerobic fat burning (Newton 2008), while protein and carbohydrates are needed to augment fat use (Lindström and Piersma 1993; Jenni and Jenni-Eiermann 1998; Piersma et al. 1999; Bauchinger and Biebach 2001). In order to accumulate energy efficiently, migratory birds also change their diet, feeding behaviour and activity rhythms (Bairlein 2002; McWilliams et al. 2004). During the migration period, physiological and behavioural adjustments also take place. Migrating birds may flexibly reduce and increase their digestive system along the migration route, depending on whether they are actively migrating or stopping over (Weber and Hedenström 2001; Ramenofsky and Wingfield 2006; Hedenström 2008). Before takeoff there is an increase in the size of heart and flight muscles, needed for efficient flight, while organs which are

not vital during the migration flight, such as stomach, intestine and liver, may shrink to reduce the flight mass (Battley and Piersma 1997; Piersma and Lindström 1997; Piersma 1998; Piersma and Gill 1998; Piersma et al. 1999; Schwilch et al. 2002; Bauchinger et al. 2005). The energy stores are usually acquired over a short time (4–10 days) just prior to migration or takeoff for the next long flight leg (Newton 2008), since excessive body mass may deteriorate flight ability, increase energy expenditure (Lindström and Alerstam 1992; Witter and Cuthill 1993; Witter et al. 1994; Schmidt-Wellenburg et al. 2007), and increase vulnerability to predators due to reduced takeoff ability and manoeuvrability (Blem 1975; Lindström and Alerstam 1992; Lima 1993; Witter and Cuthill 1993; Witter et al. 1994; Kullberg et al. 1996; Lind et al. 1999; Burns and Ydenberg 2002).

1.2. Energy regulation and stress in birds

1.2.1. Corticosterone secretion

Behavioural and physiological changes necessary to cope with different environmental challenges, predictable or unpredictable, are elicited by various hormones. Corticosterone is one of the main stress hormones, as well as the primary energy-regulating hormone in birds (Holmes and Phillips 1976; Harvey et al. 1984; Norris 2007). Plasma corticosterone seems to operate at three distinct levels: 1) a low baseline level, which supports the most fundamental requirements of life; 2) an elevated seasonal baseline level, which varies with predictable changes during the annual cycle (migration, breeding and overwintering); and 3) a high acute stress-related level (adrenocortical response), which helps individuals survive unpredictable life-threatening situations such as predation and infections (Wingfield et al. 1998; Holberton 1999; Landys et al. 2006). Regardless of the stressor, the hormone-based stress response appears to be common in the vast majority of vertebrates. In a stressful situation, the rapid release of catecholamines, such as dopamine, adrenaline and noradrenaline, is followed by activation of the hypothalamic-pituitary-adrenal axis (HPA) (Harvey et al. 1984; Wingfield 1994; Wingfield et al. 1997; Buchanan and Evans 2000). This HPA activation causes an elevation of the corticosterone concentration in the blood within 2–3 minutes, reaching peak levels within 30–60 min after the disturbance (Wingfield 1994; Holberton 1999; Canoine et al. 2002). The release of corticosterone, at either the baseline or an acute stress level, promotes gluconeogenesis, the biosynthesis of carbohydrates from non-carbohydrate precursors, and thus causes an elevation of the blood glucose concentration, which is important for example in promoting pre-migratory fuelling (Norris 2007).

Physiological stresses in free-living wild birds can be studied with a standardised stress-protocol, in other words capture and handling, which according to Wingfield et al. (1992) are perceived by an organism as a life-threatening situation. A short-term increase in the corticosterone level may help the individual in an acute life-threatening situation; chronically elevated corticosterone concentrations, on the other hand, may cause damage to the immune system, protein loss in skeletal muscles, and the suppression of growth and development (Wingfield 1994; Wingfield et al. 1997; Holberton 1999; Buchanan and Evans 2000; Sapolsky et al. 2000). Birds may decrease their adrenocortical response in order to reduce these long-term negative effects, for example when its expression might compromise reproductive success, or conversely may increase it when a quick response is needed (Wingfield et al. 1995; Norris 2007).

Baseline corticosterone levels and the magnitude of the adrenocortical response in birds may be modified by such factors as age, sex, season, breeding stage, nutrition state, body condition, habitat quality and weather conditions (Astheimer et al. 1992; Wingfield et al. 1994; Romero et al. 1997; Suorsa et al. 2003; Jenni-Eiermann et al. 2008). Weather conditions can affect energy resources, thus also affecting baseline and stimulated corticosterone levels (Astheimer et al. 1995; Smith et al. 1994; Wingfield and Ramenofsky 1997; Romero et al. 2000). Birds may not find enough food and/or they may need more energy for thermoregulation in bad weather, which leads to decreased fat resources. This in turn increases the baseline corticosterone level and reduces the stress-induced corticosterone response (Wingfield et al. 1982; Rogers et al. 1993; Smith et al. 1994; Wingfield and Ramenofsky 1997; Jenni-Eiermann et al. 2008).

1.2.2. Migration modulation hypothesis (MMH)

Many studies have found moderately elevated levels of baseline corticosterone in different bird species during the migration period (Holberton et al. 1996; Romero et al. 1997; Piersma and Ramenofsky 1998; Holberton 1999; Piersma et al. 2000; Landys et al. 2004a; Landys et al. 2004b; Agatsuma and Ramenofsky 2006; Holberton et al. 2007; Holberton et al. 2008). According to the migration modulation hypothesis (MMH), this is related to the development and/or maintenance of migratory condition (Holberton et al. 1996; Holberton 1999; Long and Holberton 2004). Moderately elevated baseline corticosterone levels increase glucose in the blood (Norris 2007), which in turn increases searching behaviour, the food intake rate (hyperphagia) and fat stores (Astheimer et al. 1992; Breuner et al. 1998; Wingfield et al. 1998; Löhms et al. 2003; Dallman et al. 2004; Landys et al. 2004a; Landys et al. 2004b; Long and Holberton 2004; Löhms et

al. 2006; Holberton et al. 2008). If an increase in baseline corticosterone concentration is prevented, birds do not fuel for migration (Landys et al. 2004b; Holberton et al. 2007).

According to the MMH, the acute adrenocortical stress response may also be reduced in order to protect skeletal muscle proteins from corticosterone-induced degradation during up-regulated baseline corticosterone levels (Holberton et al. 1996; Holberton 1999; Long and Holberton 2004). Baseline levels of corticosterone do not remain elevated throughout the whole migratory period, but can vary substantially depending on the stage of the migration (i.e. takeoff, flight, landing, stop-over) and/or the energetic condition of the bird (Holberton 1999; Piersma et al. 2000; Landys-Ciannelli et al. 2002; Long and Holberton 2004; Falsone et al. 2009). The MMH has been tested with different bird species both in the field and the laboratory. Some recent studies with warblers, sparrows and shorebirds have supported the MMH (Wingfield et al. 1994; Holberton et al. 1996; Romero et al. 1997; Piersma and Ramenofsky 1998; Holberton 1999; Piersma et al. 2000; Landys-Ciannelli et al. 2002; Landys et al. 2004a; Landys et al. 2004c; Long and Holberton 2004), but the results are not consistent across all species and seasons (Schwabl et al. 1991; Gwinner et al. 1992; Holberton et al. 1996; Jenni et al. 2000; Romero et al. 1997; Tsipoura et al. 1999).

1.3. Redox state and biotransformation enzyme activity

In addition to adrenocortical stress, long-distance migratory birds may also suffer from oxidative stress, partly due to increased energy turnover during migration. Normal metabolic activity (mitochondrial processes), as well as exposure to environmental perturbations such as radiation, xenobiotics (exogenous chemicals), toxins, air pollutants, metals, various biotic or abiotic stresses and diseases etc., produce reactive oxygen species (ROS), which may be harmful to organisms (Scandalios 1997; Scandalios 2005). A certain level of ROS is necessary in maintaining a redox state, which is determined by the balance between oxidants and antioxidants (Beckman and Ames 1998; Finkel and Holbrook 2000; Halliwell and Gutteridge 2007). Antioxidants can be defined as substances that significantly delay, prevent or remove oxidative damage to molecules (Halliwell and Gutteridge 2007). When the redox state is shifted in an oxidative direction (oxidative stress), the formation of various reactive oxygen species (ROS) exceeds the organism's antioxidant defence capacity; this initially impairs cellular signalling dependent on ROS, and at more severe levels causes damage to biomolecules, such as DNA, proteins and lipids (Beckman and Ames 1998; Finkel and Holbrook 2000; Valavanidis et al. 2006; Halliwell and Gutteridge 2007; Monaghan et al. 2009). At a certain level, however,

oxidative stress is always present, since pro-oxidants are continuously produced in normal mitochondrial processes (Pamplona and Costantini 2011).

In birds oxidative stress may cause individual variation in breeding success, life span, activity and many functional responses, such as the immune response (Costantini 2008). In the case of long-distance migrating birds in particular, increased energy expenditure, extensive fuelling and prolonged depletion of fats and proteins, which require specific adaptations in order to maintain body homeostasis, may pose a threat of oxidative stress (McWilliams et al. 2004; Costantini et al. 2007). In addition, the varying chemical quality of the food available at different life-history stages also exposes long-distance migrating birds to different toxic compounds, whether naturally produced or of human origin. The amount of oxidative stress in long-distance migrants may vary seasonally (McWilliams et al. 2004; Costantini et al. 2007).

To avoid the physiological damage caused by oxidative stress or toxic compounds, organisms have evolved a high diversity of sophisticated enzymatic or non-enzymatic systems using antioxidant compounds. The enzymes involved in regulating the redox balance include glutathione-S-transferase (GST), glutathione peroxidase (GP), glutathione reductase (GR), superoxide dismutase (SOD) and catalase (CAT) (Halliwell and Gutteridge 2007; Costantini 2008; Monaghan et al. 2009; Koivula and Eeva 2010). Small antioxidant molecules involved in the regulation of the redox balance may be obtained via food (carotenoids, flavonoids, and vitamins E and C), or may be produced endogenously, as in the case of glutathione (GSH), the major small molecule regulating the redox-state (Halliwell and Gutteridge 2007; Costantini 2008; Monaghan et al. 2009).

Oxidative stress is often associated with changes in biotransformation activity (Halliwell and Gutteridge 2007). With biotransformation systems most xenobiotics can be metabolized by converting them into a more soluble form, which is therefore easier to excrete (Halliwell and Gutteridge 2007). Compounds which activate the aryl hydrocarbon receptor (AhR) and the consequent xenobiotically induced gene expression, including biotransformation enzymes of the cytochrome P450-family (CYPs), cause the formation of oxygen free radicals (e.g. Whyte et al. 2000). Ethoxyresorufin-*O*-deethylase (EROD) enzyme activity usually determines the activation of AhR and its activity can be easily measured (Lorenzen et al. 1997). The parameters used in the biomonitoring of birds may change at different stages of the bird's life cycle. Seasonal physiological processes, such as moult (Franson et al. 2002), reproduction (Alonso-Alvarez et al. 2004; Wiersma et al. 2004; Bertrand et al. 2006) and heavy exercise during migration cause variation in the redox state in both wild and captive birds (Costantini et al. 2007; Costantini et al. 2008; Larcombe et al. 2008). If we want to assess the oxidative stress or

biotransformation activity caused by chemical exposure, we need to understand normal (natural or physiological) seasonal changes in redox parameters, including the relevant enzyme activities (e.g. Stohs and Bagchi 1995).

1.4. Aims of the thesis

During past decades, barn swallow populations have declined over large areas in Europe (Møller 1989; Tucker and Heath 1994; Robinson et al. 2003). The reasons for this decline are not completely clear, but their survival and breeding success has been affected by human activity in breeding, migration and wintering areas. The barn swallow population decline has been ascribed to a lack of suitable breeding areas (Tucker and Heath 1994); intensified agriculture (Tucker and Heath 1994; Donald et al. 2001); decreased dairy farming (Møller 2001; Ambrosini et al. 2002a; Ambrosini et al. 2002b); increased use of pesticides (Vickery et al. 2001; Benton et al. 2002) and climate change, especially in wintering areas (Møller 1989; Tucker and Heath 1994). Population declines in migratory birds seem to be substantial in species breeding in farmland in northern areas and wintering in sub-Saharan Africa (Møller et al. 2008). The barn swallow is one such species; thus, identifying the factors affecting survival in the annual cycle may lead to a better understanding of the processes of population dynamics and the evolution of migration.

The purpose of this thesis is to study the seasonal variation (with-in season and between-seasons) in migratory body condition, corticosterone secretion, oxidative stress and biotransformation activity of barn swallows (*Hirundo rustica* L.). These factors cause behavioral and physiological changes during long-distance migration (e.g. timing, fuelling, body condition, biotransformation of toxics), which are not only directly associated with the success of migration, but also affect other phases in the annual cycle. Recent studies have shown that for example corticosterone secretion is associated with measures of fitness in many bird species (Bonier et al. 2009). In the first article (**I**) I use Finnish Ringing Centre data collected during 1997–2009, initially as a part of the EURING Swallow Project conducted during 1997–2002. The idea of a project was to collect field data by ringing barn swallows in Europe, Africa and Asia in their breeding and wintering areas as well as along their migration routes (roosts). These data make it possible for example to study barn swallow's fuelling along the migration route and to test different migration theories. In Finland the active ringing of barn swallows extended beyond 2002, producing a large number of recaptures at the night roosts of individual barn swallows ringed as nestlings. My purpose in article **I** is to analyse temporal changes in the directions of movement of young birds, so as to identify the actual start of the

autumn migration. An additional purpose is to determine the extent to which juvenile barn swallows accumulate fat and increase body mass before autumn migration, and whether there is a difference in fuelling behaviour between juveniles from first and second broods. I also want to observe how juvenile age and migration distance from the nest are associated with fat accumulation and mass increase. Such information can help in interpreting findings concerning physiological regulation related to premigratory/migratory stage, i.e. energy accumulation and corticosterone secretion. Due to the seasonal constraints in the autumn, juveniles from second broods may need to prepare for migration faster than first brood ones. In autumn 2003 I captured barn swallows arriving at their night roosts and held them in captivity overnight (**II**). My purpose is to determine the possible relationship between on the one hand the progress of autumn (date) and/or ambient temperature, on the other the increase in body mass and the accumulation of fat, overnight body mass loss, overnight fat loss, and faecal production. These two articles (**I, II**) focus on migratory fuelling prior to the autumn migration and its during early stages; they help to understand the factors associated with movement direction, timing and fuelling behaviour before and during migration. The analyses of movement direction and the initiation of autumn migration (**I**) is intended to ensure that the barn swallows we sampled for the studies of corticosterone and oxidative stress were already migrating, i.e. moving in the true migration direction (180 °).

Conservation biologist have already long made use of field endocrinology protocols in measuring stress in free-living birds, since these protocols can provide reliable information on both natural and human-induced environmental stresses (Walker et al. 2005), and the results of such studies are feasible for conservation biology (Smith et al. 1994; Wingfield et al. 1997; Wikelski and Cooke 2006; Wingfield 2008). Over recent decades, the determination of hormones from very small blood samples has become routine. With blood samples, the measurement of corticosterone, the major energy-regulating and stress hormone in birds (Harvey et al. 1984; Holmes and Phillips 1976; Norris 2007) has also become the standard way of assessing the level of stress that birds are exposed to. Data for the baseline and stress-induced levels of corticosterone in the third and fourth articles (**III, IV**) were collected in Finland (autumns 2006, 2007, 2011) and in South Africa (winter 2007). The main purpose of the third article (**III**) is to study baseline and stress-induced levels of corticosterone before autumn migration in relation to the energetic condition of a bird (mass, fat score), the progression of autumn (date) and ambient weather conditions (temperature, rainfall). In the fourth article (**IV**) I compare differences in baseline and stress-induced corticosterone secretion prior to the spring and autumn migrations, to determine whether barn swallows express seasonal differences in corticosterone secretion, and thus in energy accumulation.

In the fifth article (V), the focus is on between-season (annual cycle) variation in the redox state and in biotransformation enzyme activity. It is important to understand normal seasonal changes in redox parameters in order to estimate the oxidative stress caused by possible chemical exposure. I use several biomarkers to assess the amount of oxidative stress and biotransformation activity in barn swallows. The biomarkers studied are EROD, GST, totGSH, GSH/GSSG-ratio, SOD, CAT, GP, GR (explained earlier in 1.3), lipid hydroperoxide (LHP) and glucose-6-phosphate dehydrogenase (G6PDH). I also measure the extent of oxidative damage to lipids; LHP can be used as a possible sign of oxidative damage, since when the damage is extreme there may be harm to other biomolecules as well, such as DNA, proteins and lipids (Beckman and Ames 1998; Finkel and Holbrook 2000; Valavanidis et al. 2006; Halliwell and Gutteridge 2007; Monaghan et al. 2009). G6PDH is an important enzyme in producing NADPH, which is needed in some detoxification pathways, and it works as a cofactor with antioxidant enzymes such as GR and CAT (Halliwell and Gutteridge 2007). EROD and GST enzymes are important in the detoxification of various xenobiotics. When detoxification does not succeed or is less efficient, the level of oxidative stress in birds may rise. Level of enzymes (EROD, GST), and the amount of antioxidants (GSH and enzymatic antioxidants) can be measured from samples of liver tissue.

2. MATERIAL AND METHODS

2.1. *Study species*

The barn swallow is a small, insectivorous passerine bird with a vast geographical range. Palearctic populations of barn swallows breed all over Europe and winter in sub-Saharan Africa. The body mass of barn swallows varies from 16 to 20 g, and may increase up to 25–26 g as a result of fuelling (Cramp 1988; Møller 1994; Turner 2006). Barn swallows breed either in colonies or in single pairs in many different habitats, but they readily choose human dwellings close to farmland, such as barns and cattle stalls, which provide suitable sites both for feeding and nesting (von Haartman 1969; Cramp 1988; Turner 2006). In the years 1997–2009 the mean egg laying date in southern Finland varied between 3–14 June \pm 5.6–10.1 d (mean \pm sd; calculated from data of the Finnish Ringing Centre). Clutch size varies between 4–7 eggs and the female incubates for 14–16 days, after which the nestlings are reared by both parents (von Haartman 1969; Møller 1989; Møller 2002). Juveniles fledge at the age of 17–21 days (von Haartman 1969). In Finland about 10–20 % of females lay a second clutch (von Haartman 1969). The second or third clutches are usually smaller than the first one (Grüebler and Naef-Daenzer 2008a; Grüebler and Naef-Daenzer 2008b; Møller 1994; Møller 2002). In Denmark the average brood size at fledging was 4.14 (SE = 0.06) in first broods and 3.69 (SE = 0.07) in second broods (Møller 1994). In Switzerland average brood size at a corresponding stage was 4.39 ± 1.14 (SD) in first broods and 3.21 ± 1.80 in second ones (Grüebler and Naef-Daenzer 2008a).

Barn swallows mainly forage for flying insects over waters, fields and meadows (Cramp 1988). In their breeding areas barn swallows catch large flies, but also beetles, lice, bees, mosquitoes and aphids (Cramp 1988). Foraging takes place either alone or in small flock (Møller 1994). Compared to other swallow species, barn swallows' food sources are larger and harder to catch, but their energy contents are greater (Cramp 1988; Møller 1994; Norberg 1994). Since barn swallows forage for aerial insects, bad weather may reduce their food availability.

The barn swallow is a long-distance migratory bird, and populations breeding in Sweden, Finland, the Baltic countries and further east are most likely to overwinter in the eastern part of South Africa, for instance in the Johannesburg area (Szép et al. 2006; Szép et al. 2007; Ambrosini et al. 2009). Barn swallows are in principle able to feed while migrating, although they also accumulate mass and fat for the migration (Pilastro and Magnani 1997; Rubolini et al. 2002). In different parts of Europe energy accumulation for the migration

starts at various times in late August or early September, and it may be less pronounced in the north than in the south, supposedly because ecological barriers at northern latitudes are less demanding (Fransson et al. 2001; Kullberg et al. 2003). Barn swallows in northern European areas (Scotland, Scandinavia, northern Russia, the Urals) may set off on their autumn migration as much as a month earlier (late August – September) than those in southern Europe (September – early October; Turner 2006), partly because of the earlier deterioration of ecological conditions, including weather conditions, and consequent decrease in the numbers of aerial insects (Ormerod 1989). During migratory fuelling the mean fat load of barn swallows may increase up to 30–40 % of lean body mass (Pilastro and Magnani 1997; Rubolini et al. 2002). Such energy stores may enable an uninterrupted migration of over 3000 kilometres, as estimated using Pennycuick’s range programs (Pennycuick 2008), which is important when crossing wide ecological barriers such as the Mediterranean or the Saharan desert (Pilastro and Magnani 1997; Rubolini et al. 2002). According to ringing recoveries, northern and eastern populations most likely migrate via the Balkan Peninsula, which is probably the most important autumnal fuelling place for Finnish barn swallows (Finnish Ringing Centre; Figure 1).

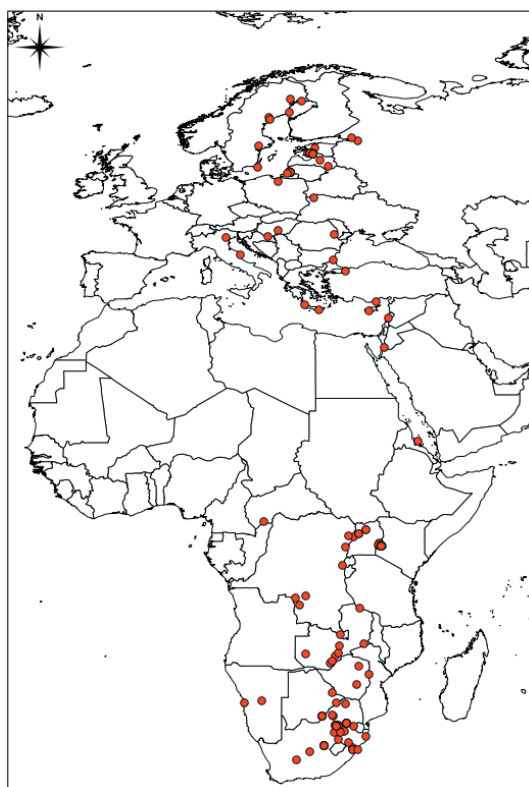


Figure 1. Barn swallows ringed in Finland and recaptured abroad (1933–2009).

2.2. Bird capturing and study areas

During the first days after fledging barn swallow juveniles usually roost near the nest, but quite soon after independence they join the communal roosts in nearby reed beds (Møller 1994). This communal roosting behaviour is typical of barn swallows. Birds start to gather around the roost an hour or more before sunset; finally, at dusk, they form tighter flocks and plunge down in small groups (Turner 2006). They will leave the roosts at sunrise. It may be that this synchronous behaviour, in large numbers and with rapid, high flight before entering and after leaving the roost, makes it difficult for predators to catch individual birds (Bijlsma and van den Brink 2005). Due to this roosting behaviour, however, barn swallows are quite easy to capture, making them a good study species for the evaluation of physiological regulation and fuelling during migration. In article I I used two different sets of data collected in Finland: 1) all juveniles ringed from nests during 1997–2009 ($n = 56497$; Figure 2a), and 2) all juveniles ringed first as nestlings and recaptured from night roosts later in the same autumn ($n = 1869$; Finnish Ringing Centre; Figure 2b). I used the first data set to identify the annual timing of first and second broods, which was essential for the analyses of fuelling behaviour and migration movements with the second data set (I).

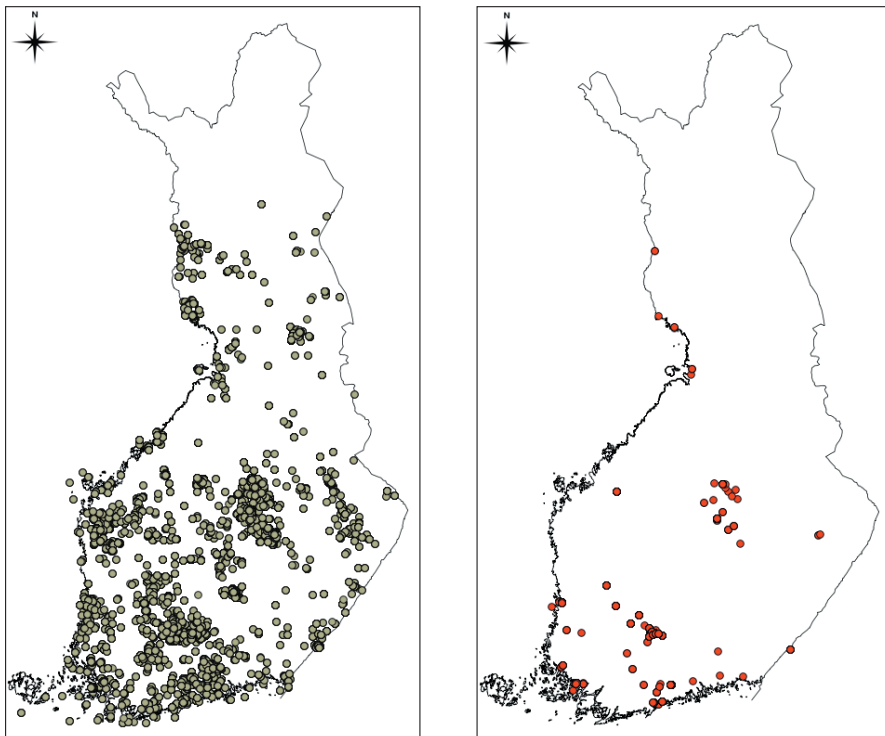


Figure 2. Ringing sites of barn swallow nestlings and recapture sites of juvenile barn swallows in Finland in 1997–2009.

I captured the barn swallows before autumn migration (**II–V**) in SW-Finland, Kuusisto (autumn 2003; 60° 24'N, 22° 30'E), Friskala (2003, 2006 and 2007; 60° 24'N, 22°15'E) and Petteby (2007 and 2011; 60° 17'N, 22° 11'E) when the birds settled down at their overnight roosts. In spring and summer 2007 the barn swallows were captured at Petteby (**V**). I tape-lured and mist-netted the barn swallows from the roosts during sunset, around 17:50–21:30 pm. (2003, 2006, 2007), and after sunrise, around 4:30–8:30 am. (2011). I ringed and measured individuals for maximum wing length (Svensson 1992), longest and shortest tail feathers (adults), fat score (ranging from 0 = no subcutaneous fat to 8 fat layer covers the ventral side completely; Kaiser 1993), and body mass (measured to the nearest 0.1 g with a spring balance). The ringing procedure was always performed the same way, accurate measurements make it possible to estimate the mass increase and accumulation of fat during migratory fuelling in autumn (**I–IV**). In 2007, barn swallows were captured before spring migration at Potchefstroom (approximately 100 km SW of Johannesburg), South Africa (26°42'S, 27°06'E) using the same procedure as in Finland (except the tape-luring), about 0.5–3 hours after sunrise at 6:30–8:00 am.

2.3. Blood sampling and measuring of birds

In order to measure baseline levels of corticosterone, I collected the first samples of blood within 2–3 minutes after capture. These samples are known to reflect the baseline corticosterone concentrations (Wingfield et al. 1982; Sapolsky et al. 2000; Romero and Reed 2005). I took the second blood sample 30 minutes later and the third 60 minutes after capture, to assess the hormonal response to capture and handling stress (**III–IV**).

Before the second blood sample I ringed and measured the birds. I determined the age of the barn swallows according to Jenni and Winkler (1994) and Svensson (1992). Blood sampling and measurement were performed the same way in South Africa and in Finland. Plasma corticosterone concentrations were determined using Correlate-EIATM corticosterone enzyme immunoassay kits (Assay Designs, Ann Arbor, MI, USA). The samples were analysed in batches as duplicates.

To be able to compare the differences in corticosterone secretion prior to the spring and autumn migration, I needed to estimate the timing of migration in South Africa and in Finland. The spring migration at Gauteng (Johannesburg area) started around 12 March; the midpoint of departure was 3 April (Altwegg et al. 2012). In Finland, the midpoint of departure in 2000–2004 was at the beginning of September (P. Suorsa, unpublished data). The first blood samples were thus taken about 5–6 weeks before the median date of migration in both South Africa and Finland (**IV**).



Figure 3. Captured barn swallows in the mist nets (Friskala, SW Finland, 24.8.2006 at 19:00).



Figure 4. Blood sampling of juvenile barn swallow at the autumn roost (Friskala, SW Finland, 14.9.2006 at 18:25).

2.4. Liver sampling in Finland and in South Africa

After taking the blood samples we killed the barn swallows by cervical dislocation, pressing the trachea with the thumb to make sure of a quick death (only in 2007). We dissected the carcasses and collected liver samples (V), placing the samples immediately in liquid nitrogen. The remainder of each carcass was frozen (initially at -5°C ; subsequently maintained at -20°C) for further analyses. The samples from South Africa were shipped to Finland by air, in dry ice. In 2007 the sex of the barn swallows was confirmed by examining the gonads. The parameters were measured using 96-well and 384-well microplates, which in most cases required reducing reagent volumes compared with the volumes recommended in the method instructions. All measurements were performed with an Envision plate reader (Perkin-Elmer) except for protein content determinations, which were performed with a Victor 1 plate reader (Perkin-Elmer).

2.5. Weather data

Weather data (II and III), including the mean temperature and rainfall of the sampling day and the day prior to sampling, were based on measurements of the Finnish Meteorological Institute in Turku ($60^{\circ}45'\text{N}$, $22^{\circ}18'\text{E}$). For article IV weather data were obtained from the Weather Station at Potchefstroom, North-West Province, South Africa ($26^{\circ}73'\text{S}$, $27^{\circ}06'\text{E}$). I used the data to study associations between weather and fuelling or weather and corticosterone secretion.

2.6. Data analysis

I used residual mass (deviation from expected body mass based on mass-wing length regression) (mass in article II) and fat score as measures of body condition. Residual mass also takes into account non-lipid energy sources, such as muscles, and is therefore a more general measure of condition than the fat score (Long and Holberton 2004). I tested the timing of southward flight from the nest, indicating the start of migration (I), with Oriana circular statistics from Kovach Computing Services (Kovach 2011). In the analyses I used single sample distribution tests, such as Rayleigh's test of uniformity and Rao's spacing test (Kovach 2011). I analysed most of the data using general/generalized linear models (GLM/GzLM) with the GLIMMIX- (I), MIXED- (II) or GENMOD-procedure (III-IV) of SAS (version 9.2; SAS 2008). I also used principal component analysis (PCA) with Predictive Analytics Software statistics 18.0 (V). Generalized

linear models (GzLM) fit the model to data by maximum likelihood estimation of the parameters. GzLMs make it possible to select an appropriate link function and response probability distribution for the model; the models are thus not sensitive to skewed data. I used a negative binomial or lognormal distribution with log link function for non-normally, and a normal distribution for normally distributed data. The fit of the models to the data (**III–IV**) was evaluated with values of scaled deviance, which is the residual deviance for the model (the sum of individual deviance contributions). The optimal value for the goodness of fit is 1 (SAS 2008).

3. MAIN RESULTS AND DISCUSSION

3.1. *Migratory fuelling and southward movements of juvenile barn swallows (I–II)*

The start of autumn migration differed between juveniles from first and second broods in terms of timing and age. Juveniles from first broods started heading south after mid-August, while only a small proportion of juveniles from second broods had moved a greater distance before mid-September. Juveniles from first broods directed their movements southward at an age of 53 days, while juveniles from second broods did so at an age of 42 days or even less. Juveniles may move away from their nest site after reaching independence, and they apparently tend to wander around rather than start the migration (Turner 2006). In our data, this was especially true for juveniles of first broods. Since moving to an unknown area may incur costs, it may be profitable to prolong the stay near the breeding grounds as long as possible.

Body mass and fat score increased during the autumn, suggesting that barn swallows do fuel for the initial stages of their autumn migration (**I–III**). The mass increase of juvenile barn swallows during the autumn was 8 %; the mean fat load was 15 % of the lean body mass (**II**). This is quite similar to the values (mean fat load 19 %) measured in southern Europe before the autumn migration, where the heaviest barn swallows had fat loads of 30–40 % of their lean body mass (Pilastro and Magnani 1997; Rubolini et al. 2002). It may be that barn swallows do not need to build up maximal energy stores during the early stages of the autumn migration, since (unlike many land birds or shorebirds) they are able to feed while flying. The ecological barriers in northern Europe may also not be particularly harsh from the point of view of barn swallows. Barn swallows may not experience complete fasting at all during their migration flight. However, the distribution of aerial insects along the migration route in autumn may be irregular and probably unpredictable, due to variable foraging habitats and changing weather conditions (Grüebler et al. 2008). Thus barn swallows too need to accumulate some energy already in the north.

Juveniles from second broods were in better body condition (residual mass, fat score) than juveniles from first broods at corresponding ages (**I**). Juveniles from second broods also had higher fat scores irrespective of age. Since the sizes of second or third broods in barn swallows are usually smaller than that of the first brood, parents may have been able to provide more food for the juveniles of second broods (Grüebler and Naef-

Daenzer 2008a; Gruebler and Naef-Daenzer 2008b; Møller 1994; Møller 2002). Another possibility is that the parents provided shorter post-fledging care for the first than for the second brood (Gruebler and Naef-Daenzer 2008a; Gruebler and Naef-Daenzer 2008b). Longer parental care may improve both body condition (Lind 2004) and the skills needed to survive (Marchetti and Price 1989; Wheelwright and Templeton 2003; Yoda et al. 2004). As Gruebler and Naef-Daenzer (2008b) have suggested, juveniles from second broods may be in better body condition already when leaving the nest. Then again, only about 20 % of parents in Finland breed twice. The difference between broods may in part be due to the fact that juveniles are usually moderately heavy before leaving the nest (their body mass can be higher than that of the adults) (Turner 2006). These high chick masses before fledging are typical for aerial insectivores (Turner 2006). On the other hand, it may well be that juveniles from first broods in prime body condition had already started the migration and thus left the country. A check of the ringing data of recaptures along the migration route south of Finland (Figure 1) offered no evidence of recaptured juveniles in good condition in early August.

Before their autumn migration, barn swallows seem to make physiological adjustments in order to preserve the energy already accumulated for migration. This can be deduced from the decreased proportional overnight mass loss, fat loss and faecal production in the course of the autumn (II). A number of studies have found that the size and function of the digestive system lessens before and during migration (Hume and Biebach 1996; Battley and Piersma 1997; Piersma and Lindström 1997; Piersma 1998; Piersma and Gill 1998; Piersma et al. 1999; Schilch et al. 2002; Bauchinger et al. 2005). This decrease in internals reduces the flight mass and may also reduce basal metabolic rate. Then again, during migratory fuelling the birds' energy utilization efficiency increases due to increased gut size (increases assimilation efficiency), and can thus reduce the amount of faeces, thus facilitating fat accumulation (Bairlein 1985; Hume and Biebach 1996). Depending on whether migrating birds are actively migrating or stopping over, they may repeatedly reduce and increase their digestive system along the migration route (Weber and Hedenström 2001; Ramenofsky and Wingfield 2006; Hedenström 2008).

The ambient temperature did not seem to affect the overnight fat loss of barn swallows, but it was positively related to the proportional overnight mass loss and negatively to faecal production (II). Goymann et al. (2006) observed in their study of wintering European stonechats (*Saxicola torquata*) that when ambient temperature was low, the birds ate more and produced more droppings. This is what we found as well; the warmer the weather, the less barn swallows defecated. As the autumn progressed, the birds

reduced their faecal production. The birds were apparently trying to preserve the energy already fuelled.

3.2. Corticosterone secretion (III–IV)

Interestingly, corticosterone does not seem to be at all involved in the autumn fuelling of barn swallows, since the baseline corticosterone levels were not elevated during the autumn (III–IV). In fact they were very low in general. Temperature and rain had only a marginal effect on the variation in corticosterone secretion, which is interesting given the potential impact of the weather on the ability of barn swallows to feed on aerial insects (III). The baseline corticosterone levels were slightly increased before the spring migration compared to the levels before autumn migration (IV), but elevated levels were positively associated with residual mass. If high baseline corticosterone levels induce fuelling, one would expect body condition to be negatively associated with baseline levels. The baseline corticosterone levels in South Africa, however, were 2.6 times higher than in Finland. In earlier studies with red knots (*Calidris canutus*), American redstarts (*Setophaga ruticilla*) and white-crowned sparrows (*Zonotrichia leucophrys*), researchers similarly found elevated baseline corticosterone values in spring compared to those in autumn (Romero et al. 1997; Marra and Holberton 1998; Piersma and Ramenofsky 1998). The African barn swallows were still moulting, and according to Romero (2002) the baseline levels should then be at their lowest. I did not, however, find any relationship between moult and baseline corticosterone levels.

There may be several reasons explaining the relatively high levels of baseline corticosterone in South Africa. First, foul weather is known to increase the secretion of baseline corticosterone and to decrease the stress-induced adrenocortical response (Wingfield et al. 1982; Rogers et al. 1993; Smith et al. 1994; Wingfield and Ramenofsky 1997; Jenni-Eiermann et al. 2008). When it rains, insects do not fly and the body mass of barn swallows may decrease (Ormerod 1989; Pilastro and Magnani 1997; Raja-aho 2005; Jenni-Eiermann et al. 2008). Then again, drought may reduce the amount of aerial insects. Weather conditions during the capture period in South Africa were quite optimal; warm and no rain. Our sampling site near the river has large numbers of insects even during dry spells, so the barn swallows were probably not suffering from hunger prior to the spring migration. The likely reason for the elevated baseline levels of corticosterone in these barn swallows seems to be the increased activity of biotransformation enzymes and high oxidative stress (IV). It is even possible that corticosterone does not promote the fuelling of barn swallows at all.

The spring migration of barn swallows may be more demanding than that of the autumn, since in spring early arrival and good body condition are crucial for good breeding success (Møller 1994). Barn swallows may migrate almost twice as fast in spring as in autumn (Mead 1970; Mead 1983; Newton 2008). Poor weather and food conditions during wintering may have a negative impact on body condition and thus reduce the speed of moult, which is associated with slow fat accumulation and delayed departure from the wintering area (Saino et al. 2004a). In years of poor ambient conditions in the wintering areas, returning barn swallows are in poor body condition, start breeding later and lay smaller clutches than in years when wintering conditions are good (Saino et al. 2004b). Success in spring migration thus seems to have direct consequences for fitness.

Before the autumn migration barn swallows responded strongly to acute capture and handling stress by increasing their corticosterone levels, regardless of whether capture took place in the evening or the morning (III–IV). I also observed that barn swallows in good body condition clearly suppressed the adrenocortical response. This is the second prediction of MMH: during the migratory period, adrenocortical response can be reduced so as to protect skeletal muscles from the hormone's catabolic activity (Holberton et al. 1996; Holberton 1999; Jenni et al. 2000). The adrenocortical response of barn swallows prior to the spring migration was also suppressed, possibly because of already elevated baseline corticosterone levels.

3.3. Oxidative stress and biotransformation activity (V)

The parameters indicating biotransformation activity and a redox state of barn swallows differed significantly according to season. Samples used for the analyses were taken before spring migration (winter), after arriving to breeding areas (spring), during breeding (summer), and before autumn migration. Values were especially high before the spring migration. Birds that had recently arrived at their breeding grounds had similar values as birds captured during the breeding period. In general, biotransformation activity and indications of oxidative stress were high in wintering barn swallows; spring and summer birds were profiled with low biotransformation activity and intermediate oxidative damage; and autumn birds had low biotransformation activity and oxidative stress, but some of the redox enzymes (SOD, GR, and G6PDH) were highly active. Low enzyme activity and intermediate oxidative stress are apparently associated with preparation for breeding or with breeding itself (SOD, CAT and GR), while high activity of some enzymes is associated with energetic preparation for migration (SOD, GR, and G6PDH). Another possible explanation for the relatively high values found in wintering barn

swallows in South Africa may be the fact that they were captured along a river that runs through rural areas with intensive agricultural activity as well as sources of municipal waste. In addition, upriver is an area with several gold mines. Pozo et al. (2006) reported a higher PCB content in the air in South Africa than in Europe. It is thus possible that the birds captured in South Africa were exposed to higher levels of toxicants than were those captured in Finland.

4. CONCLUSIONS

In Finland, juvenile barn swallows notably increase their mass and accumulate fat before the autumn migration (**I–II**). The intensity of energy accumulation is not quite as high as in southern Europe, mainly Spain and Italy, from whence birds have both the Mediterranean and the Sahara desert to cross. By somehow improving their energy efficiency, juvenile barn swallows are apparently trying to preserve the energy already accumulated for the migration, since overnight mass loss, faecal production and fat loss all decrease in the course of the autumn. I did not, however, measure the birds' energy metabolism during overnight capture to estimate whether the energy efficiency improved during the autumn.

Interestingly, in South Africa barn swallows do not increase their mass or accumulate fat before the spring migration (**IV**), at least not during our capture period. This should be studied more carefully with more individuals and over a longer duration, since earlier studies suggest that the spring migration is all in all more demanding (faster and longer distances) than that of the autumn (Yohannes et al. 2009; Tøttrup et al. 2012;). One would expect that preparation for the spring migration would require even more pronounced fuelling. It is possible that fuelling for the spring migration takes place just a few days before departure, as excess weight may expose birds to predation due to reduced manoeuvrability and slower takeoff (Blem 1975; Lindström and Alerstam 1992; Lima 1993; Witter and Cuthill 1993; Witter et al. 1994; Lind et al. 1999; Burns and Ydenberg 2002; Kullberg et al. 1996; Schmidt-Wellenburg et al. 2007). To improve chances of surviving, suitable fuelling and roosting places, such as wetlands and reed beds, ought to be available along the migration route.

For an aerial feeder, an adaptation to convert the energy content of the prey directly to a form usable for flight could be an optimal strategy. An intriguing recent study of foraging insectivorous bats has shown them to be capable of using insect-derived nutrients immediately after consumption to fuel the bat's flight (Voigt et al. 2010): the authors conclude that the net energy gain is greater when food is combusted directly rather than first being converted into endogenous reserves. By this means bats can reduce flight costs by minimizing body mass due to stored fat. Bats benefit from saving their fat stores for times when ambient conditions are adverse, for instance during extended periods of heavy rainfall (Voigt et al. 2010). This may be an ideal feature for barn swallows as well. Whether direct food combustion is also possible for long-distance migratory birds definitely needs further research. So far, to my knowledge, it has not been studied.

In article I I show that in autumn juveniles from first broods start to move southward around mid-August, juveniles from second broods around mid-September. Juveniles from second broods have higher fat scores at the corresponding age than juveniles from first broods, which in turn may enhance their survival of migration. In addition, juveniles from second broods start migration younger than first brood ones. If the start of breeding is advanced as a result of climate change, double-brooded species will presumably be able to adjust their inter-clutch interval, which may improve breeding success due to a longer breeding season (Møller et al. 2008). In Finland the proportion of barn swallows breeding twice will perhaps increase. These positive effects will be possible only if barn swallows are able to advance the timing of both migration and breeding, so as to avoid a situation in which their prey species appear in advance of breeding (Møller et al. 2008).

Surprisingly, baseline corticosterone seems not to be associated with fuelling prior to spring or autumn migrations (III–IV). This is contrary to the first prediction of the MMH. The elevated baseline corticosterone values of barn swallows in South Africa are most likely due to biotransformation activity and high oxidative stress (V), possibly caused by toxic compounds along the river where we captured the birds. Since the biotransformation of toxic pollutants is often associated with increased energy turnover (Parkinson and Ogilvie 2008), it may also affect corticosterone secretion. What is most likely is that the toxic compounds barn swallows are exposed to in their wintering area or along the migration route increase both adrenocortical and oxidative stress (V). In evaluating the consequences of stress, the duration of the stressor is important. The effects of chronic stress in birds induced by human alteration of the environment (e.g. climate change, pollution) are often long-lasting compared to normal short-term stress, induced by predators, lack of food, or migration (Wikelski and Cooke 2006). I found that before their autumn migration barn swallows respond to stress induced by capture and handling (III–IV); in South Africa, in contrast, the response is clearly blunted, most likely due to high baseline corticosterone levels (IV). South African barn swallows could not increase the amount of corticosterone in plasma without endangering their body condition (muscle catabolism). In autumn, barn swallows in good body condition do not respond to stress as much as those in poor body condition. It may be that birds in good condition are ready to start their migration, and make physiological adjustments so as to preserve the energy already accumulated for the migration.

The intensive biotransformation metabolism of wintering birds is one likely reason explaining their oxidative stress. The low GSH/GSSG ratio, high levels of glutathione (GSH) and lipid hydroperoxides (LHPs), support this notion, and may in part explain their poor body condition (V). However, the high activities of certain enzymes (SOD,

GR, and G6PDH) are probably also associated with energetic preparation for migration, since barn swallows showed equal values for these before spring and autumn migrations. Low enzyme activities (SOD, CAT and GR) and intermediate oxidative stress, on the other hand, are associated with preparation for breeding or with breeding itself.

To conclude, I found in my thesis that for aerial feeders like barn swallows, some energy accumulation for the autumn migration takes place even if the first part of the southward migration is not overly demanding. I found no differences in fuelling rate between juveniles of first and second broods, but second broods had larger fat stores than first broods. The parents may have provided longer post-fledging care for juveniles from second broods than first brood ones, due to the limited inter-clutch interval. The main energy-regulating hormone of birds, corticosterone, did not seem to be involved in the regulation of energy accumulation. This is quite a surprising finding, and contradicts the first prediction of the MMH. The second prediction of the MMH was supported in my studies; barn swallows in good body condition (autumn) had lower adrenocortical responses than birds with poor body condition. In addition, barn swallows with elevated baseline levels of corticosterone prior to the spring migration also had a blunted adrenocortical response. I found differences between the seasons in oxidative stress and biotransformation activity: the highest levels of oxidative stress and biotransformation activity occurred in South Africa, before the spring migration. Some of the migration-related redox enzymes (SOD, GR, and G6PDH) were also high before the autumn migration, even if the birds were not suffering from oxidative stress and did not show high biotransformation activity. During preparation for breeding and the annual breeding period, the birds showed low redox and biotransformation enzyme activity.

Measures of corticosterone secretion during the breeding period would be a logical sequel to my studies, since breeding is a stressful life-history stage. It would also be important to find out how exposure to adrenocortical and oxidative stress is associated with the survival of barn swallows. In the future, new technology (e.g. satellite transmitters) may allow the more precise follow-up of individual birds, thus helping us to understand the reasons behind declining populations. In the case of the barn swallow, an understanding of their exposure to contaminants especially at the wintering grounds in South Africa would help in interpreting which changes are caused by xenobiotic biotransformation. All in all, the accumulation of xenobiotics in barn swallows should be measured.

In addition to self-sampled data (II–V), I have been able to use multi-year ringing data of a long-distance migratory bird, the barn swallow (I). This data is quite unique and rare; there are not that many long-distance passerine species that have been ringed as nestlings and recaptured the same autumn from the roosts along the migration route. In

this thesis, I have had a fascinating opportunity to explore some of the factors influencing the physiological ecology of pre-migratory barn swallows that in Finland had travelled only a few hundred kilometers – a modest distance, compared to the length of the whole journey of over 10 000 km.

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S. Raja-aho

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