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A SPORT-PHYSIOLOGICAL PERSPECTIVE ON BIRD MIGRATION: EVIDENCE FOR FLIGHT-INDUCED MUSCLE DAMAGE

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Summary

Exercise-induced muscle damage is a well-described consequence of strenuous exercise, but its potential importance in the evolution of animal activity patterns is unknown. We used plasma creatine kinase (CK) activity as an indicator of muscle damage to investigate whether the high intensity, long-duration flights of two migratory shorebird species cause muscle damage that must be repaired during stopover. In two years of study, plasma CK activity was significantly higher in migrating western sandpipers (a non-synchronous, short-hop migrant), than in non-migrants. Similarly, in the bar-tailed godwit (a synchronous, long-jump migrant), plasma CK activity was highest immediately after arrival from a 4000–5000 km flight from West Africa to The Netherlands, and declined before departure for the arctic breeding areas. Late-arriving godwits had higher plasma CK activity than birds that had been at the stopover site longer. Juvenile western sandpipers making their first southward migration had higher plasma CK activity than adults. These results indicate that muscle damage occurs during migration, and that it is exacerbated in young, relatively untrained birds. However, the magnitude of the increases in plasma CK activity associated with migratory flight were relatively small, suggesting that the level of muscle damage is moderate. Migrants may avoid damage behaviourally, or have efficient biochemical and physiological defences against muscle injury.

Key words: bird, capture stress, creatine kinase, exercise, flight, migration, muscle damage, settling time, *Calidris mauri*, *Limosa lapponica.*

Introduction

Avian migratory flight is exceptional among examples of vertebrate endurance exercise because it combines very high rates of aerobic energy metabolism with prolonged periods of activity and fasting. Flying birds consume oxygen at more than twice the aerobic limit (V_{O_2max}) of similarly sized running mammals, and migratory flights can last 50 or even 100 h (Alerstam, 1990; Butler and Woakes, 1990; Butler, 1991; Berthold, 1996). How birds budget the major resources needed for flight (particularly fat, protein and water) to migrate successfully has been the subject of much empirical and theoretical study (Piersma, 1987; Alerstam and Lindström, 1990; Piersma and Jukema, 1990; Carmi et al., 1992; Klaassen, 1996; Klaassen and Lindström, 1996; Alerstam and Hedenström, 1998; Kvist et al., 1998). Less consideration has been given to how other, non-resource-based effects of endurance flight may influence migration strategies or alter behaviour and refuelling performance at stopover sites.

In addition to refuelling, birds may undergo processes of recovery and repair at stopover sites, implying that migratory flight has costs other than the depletion of metabolic fuels and water (Klaassen and Biebach, 1994; Hume and Biebach, 1996; Klaassen et al., 1997; Piersma, 1997; Biebach, 1998; Karasov and Pinshow, 1998; Karasov and Pinshow, 2000). Frequently, birds do not gain mass in the first days after arrival at stopover sites (Alerstam and Lindström, 1990; Klaassen and Biebach, 1994; Gannes, 1999). This ‘settling time’ may represent the time required to locate good feeding areas (energy-rich and safe) or to obtain feeding territories (Rappole and Warner, 1976; Alerstam and Lindström, 1990), but settling time could also have a physiological basis (Langslow, 1976; Klaassen and Biebach, 1994; Karasov and Pinshow, 2000). For example, catabolism of the digestive system in flight may constrain hyperphagia and mass deposition after arrival (Klaassen and Biebach, 1994; Hume and Biebach, 1996; Klaassen et al., 1997; Biebach, 1998; Karasov and Pinshow, 1998; Karasov and Pinshow, 2000; Lindström et al., 1999; Battley et al., 2000).

In this study we explore the possibility that flight-induced

muscle damage is a mechanism by which the physiological side-effects of endurance flight influence migration strategies and stopover behaviour. Recent studies demonstrate that the pectoralis muscles are catabolized during endurance flights (Battley et al., 2000; Lindstrom et al., 2000). The utilization of muscle protein for metabolic fuel occurs through an alteration in the balance between the rates of protein synthesis and degradation (Goldspink, 1991). In contrast, muscle damage is a pathological consequence of strenuous exercise, especially apparent in untrained individuals (Armstrong et al., 1991; Clarkson and Sayers, 1999). The most severe damage is caused by mechanical stresses acting on muscles during eccentric exercise (stretching while active, e.g. lowering a weight; Armstrong et al., 1991; Fridén and Lieber, 1992). Prolonged, high intensity concentric exercise (e.g. cycling or running) can also lead to muscle damage, possibly as a result of metabolic factors (reactive oxygen species, elevated temperature, lowered pH, ionic shifts; Armstrong et al., 1991; Byrd, 1992; Virtanen et al., 1993; Rama et al., 1994; Havas et al., 1997; Koller et al., 1998; Fallon et al., 1999; Margaritis et al., 1999). Exercise-induced muscle damage is characterized by ultrastructural disruption (Z-line streaming), elevation of muscle-specific proteins in plasma (e.g. creatine kinase, myoglobin) and immune system responses (e.g. neutrophil infiltration; Armstrong et al., 1991; Byrd, 1992; Lieber et al., 1996; Clarkson and Sayers, 1999). Acute damage causes loss of strength, reduced mobility, pain, edema and, in extreme cases, rhabdomyolysis and kidney failure (Clarkson et al., 1992; Kuipers, 1994). Chronic damage may contribute to the immunosuppression characteristic of overtraining (Shephard and Shek, 1998). During flight, the avian pectoralis performs mainly concentric work (Biewener et al., 1998), but the high intensity and long duration of migratory flights could result in significant muscle damage. Disruption of flight muscle ultrastructure in Canada geese *Branta canadensis* upon arrival at the breeding grounds provides some of the only evidence of migration-associated muscle damage in birds (George et al., 1987).

We investigated flight-induced muscle damage during migration by measuring plasma creatine kinase (CK) activity in two species of long-distance migrant shorebirds with very different migration strategies. Plasma CK activity is one of the most widely used indicators of exercise-induced skeletal muscle damage (Clarkson et al., 1992; Morton and Carter, 1992; Komulainen et al., 1995; Sorichter et al., 1997; Clarkson and Sayers, 1999), and its suitability for use in birds is based on a number of studies (Franson et al., 1985; Bollinger et al., 1989; Dabbert and Powell, 1993; George and John, 1993; Knuth and Chaplin, 1994; Totzke et al., 1999).

The western sandpiper *Calidris mauri* is a short-hop migrant which travels non-synchronously, i.e. birds may arrive at a stopover from a multitude of departure points, and may stay for a variable number of days (Iverson et al., 1996; Butler et al., 1997). In this species, we predicted that if muscle damage occurred during migratory flights, plasma CK activity should be higher in migrants captured during stopover than in non-migrants at a tropical wintering area. We also tested for greater muscle damage in the relatively untrained juvenile sandpipers, which make their first southward migration only 6–8 weeks after hatching (Wilson, 1994).

Bar-tailed godwits *Limosa lapponica* winter in West Africa, and are thought to make a single non-stop flight of 4000–5000 km to the Dutch Wadden Sea (Piersma, 1987; Piersma and Jukema, 1990). Godwits migrate relatively synchronously, and arrive at the Wadden Sea with severely depleted body stores (Piersma and Jukema, 1990; Piersma et al., 1996). Late-arriving birds can be distinguished on the basis of feather moult (Piersma and Jukema, 1993). If muscle damage had occurred, we predicted that plasma CK activity would decline with stopover date, and always be higher in late-arriving birds.

Materials and methods

Western sandpipers

Non-migratory western sandpipers were sampled in the Gulf of Panama (8 °N, 79 °W) during pre-migratory mass gain in March, 1996, and again during the winter when low in body mass in January, 1997 (Guglielmo, 1999). Migrating sandpipers were sampled during stopover in spring (25 April–10 May) 1996 and 1997, and fall (July for adults, August for juveniles), 1996, at Boundary Bay and Roberts Bank, British Columbia, Canada (49 °10′N, 123 °05′W). Juvenile migrants were also sampled in August, 1996, at Sidney Island, a small stopover site in the southern Strait of Georgia, BC, Canada (Lissimore et al., 1999).

We captured sandpipers with mist nets (Avinet, Dryden, NY, USA) under permits from the Canadian Wildlife Service and INRENARE (Panama). Nets were in constant view, and to determine the effect of capture, blood sampling was timed from the moment of netting. Capture effect was also examined by sampling 12 birds immediately (<5 min), and again 15 min later. Approximately 40 % of the blood samples were obtained from birds killed for body composition analysis (Guglielmo, 1999). Birds were anaesthetized (Guglielmo et al., 1998) and blood was collected from the jugular vein with a heparinized Pasteur pipette. Approximately 190 additional birds were sampled from the wing by pricking the brachial vein with a sterile needle (26-gauge) and collecting blood (200–300 µl) with heparinized capillary tubes (VWR Scientific). The method of sampling (neck *versus* wing) did not affect plasma CK activity (P>0.10). Blood was transferred to heparinized 1.5 ml Eppendorf tubes (rinsed with 1000 i.u. ml^{-1} porcine sodium heparin; Sigma), and kept cool above ice. Plasma was separated by centrifugation at 6000 revs min^{-1} (2000 ***g***) for 10 min. Samples from Boundary Bay and Roberts Bank were frozen at –20 °C. In Panama and at Sidney Island, plasma was snap-frozen and transported on liquid N_2. After arrival at the laboratory these samples were also stored at –20 °C. Sex was determined by gonadal inspection (dead birds) or by culmen length measured with digital calipers (Page and Fearis, 1971). Body mass was measured (±0.01 g), and age was determined

from plumage (Wilson, 1994; P. D. O'Hara unpublished data). Protocols conformed with the Canadian Committee for Animal Care guidelines.

Bar-tailed godwits

Bar-tailed godwits were studied while en route from West Africa to breeding areas on the Taimyr Peninsula, Russia during their 1 month long stopover in the Dutch Wadden Sea (Piersma and Jukema, 1990). Early arriving godwits were captured in 1998 (29 April – 6 May) on the dunes near Castricum (52 °32′N, 04 °37′E) on the Dutch mainland, as they completed a long-distance flight from Africa (Piersma and Jukema, 1990). These birds were attracted with recorded calls and decoys, and captured in clap nets (Landys et al., 2000). In 1997 (N=142) and 1998 (N=24), refuelling godwits were captured on the island of Texel (53 °03′N, 04 °48′E) in wilsternets (Koopman and Hulscher, 1979) between 13 and 29 May. Godwits were sexed based on body size (Piersma and Jukema, 1990). At Texel, relatively late-arriving birds were identified based on the absence of body moult (Piersma and Jukema, 1993; Piersma et al., 1996). At both sites, blood sampling was timed from the moment of capture. Blood was taken by puncturing the brachial vein (23-gauge needle) and collecting the blood into heparinized capillary tubes. Samples were stored on ice, centrifuged within 10 h of collection at 6900 ***g*** for 10 min and stored at –80 °C. Plasma was transported to Canada on liquid N_2 and stored at –80 °C until analysis. Bar-tailed godwits were captured under permit from the Dutch Bird Ringing Office and blood sampled under permit from the Dutch Animal Experimentation committee.

Sample and data analysis

Creatine kinase activity was assayed on a microplate spectrophotometer as follows: 7 µl plasma and 250 µl warmed reagent (37 °C; WAKO Diagnostics, Richmond, VA, USA) in a 400 µl flat-bottomed well, were shaken for 3 min at 37 °C, and the absorbance at 340 nm was measured every 20 s for 5 min. To minimize the effects of uneven heating, the outermost wells of the plates were not used, and placement of samples was semi-randomized. We detected 70–90 % of expected CK activity in human control sera (WAKO Diagnostics). Intra- and inter-assay coefficients of variation were 9 % (N=16) and 4.6 % (N=103), respectively. Chicken plasma was run as an internal standard in each assay, and hemolyzed samples were omitted. Stability experiments indicated that storage conditions after bleeding (at room temperature 20 °C or on ice) had little effect on CK activity. Freezing and storage on liquid N_2 or at –80 °C gave the best results. Samples stored for 5 weeks at –20 °C retained 94 % of CK activity, but up to 50 % was lost by 4 months (Guglielmo, 1999). Only data from samples stored for 6 weeks or less at –20 °C, or kept at –80 °C, were used.

Creatine kinase activities were $\log_{10}$-transformed to make the data approximately normal. The effects of bleed-time and body mass were determined by regression and analysis of covariance (ANCOVA). A paired t-test was used to test for a bleed-time effect within individuals. The effects of sex, age or migratory status were tested by ANCOVA, controlling for appropriate covariates. Where needed, least-squares means were generated, and compared using a Bonferroni correction (Rice, 1989) to ensure an experiment-wise error rate of α=0.05 (two-tailed adjusted α=0.01). For western sandpipers, a *post-hoc* linear contrast was used to compare CK activity between combined migrant stages and non-migrant stages. One-tailed tests were used when we had *a priori* predictions: (1) migrant sandpipers should have higher plasma CK activity than non-migrants, (2) plasma CK activity should decline with stopover date in godwits, and (3) controlling for date, late arriving godwits should have higher plasma CK activity than early arrivals.

Results

Western sandpipers

Data from samples with bleed-times up to 20 min were used, but median bleed-time was 5 min (N=290). In the following sections, the term 'stage' refers to the combination of season, site and year (e.g. fall, Sidney Island 1996). Plasma CK activity increased rapidly with time following capture in all birds combined (F=$10.1_{1,288}$, r^2=0.26, P=0.0001; Fig. 1), as well as in every stage (P<0.02), and within individuals sampled twice, where CK activity increased about 5 % of baseline per minute (23.4±6.6 units l^{-1} min^{-1}, P=0.005). Controlling for bleed-time and stage, plasma CK activity was negatively related to body mass (F=$13.0_{1,283}$, P=0.0004) with no stage-by-mass interaction (P=0.49). In most stages the relationship between mass and CK activity was weak, but it was highly significant in juveniles sampled during their first southward migration at Boundary Bay (P=0.004) and Sidney Island (P= 0.004). Thus, bleed-time and body mass were entered into all subsequent analyses.

There was no effect of sex on plasma CK activity in any

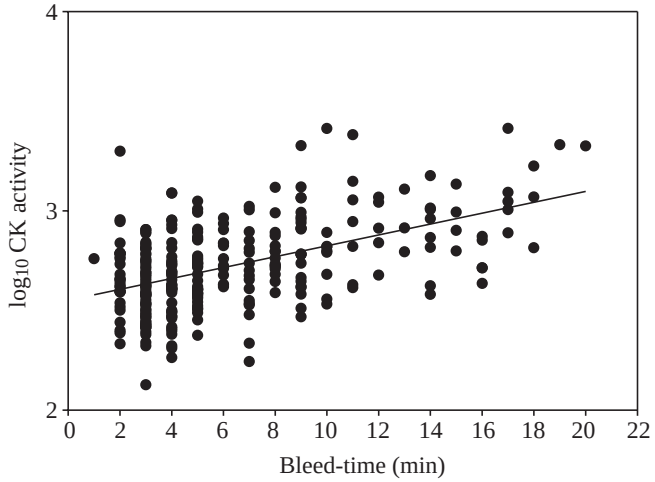

Fig. 1. The relationship between bleed-time (time after capture) and $\log_{10}$-transformed plasma creatine kinase activity in western sandpipers.

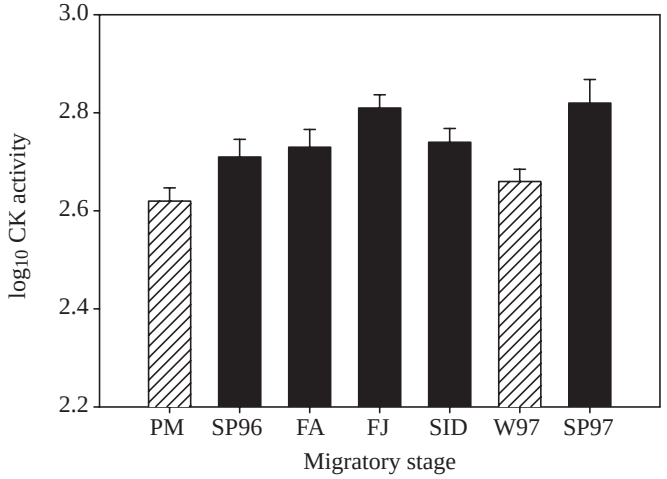

Fig. 2. Least-squares means (controlling for bleed-time and body mass) of $\log_{10}$-transformed plasma creatine kinase activity in western sandpipers collected at various stages of their annual cycle. Non-migrants are shown as hatched bars and migrants are shown in black. PM, pre-migration; SP96, spring 1996; FA, fall adult 1996; FJ, fall juvenile 1996; SID, Sidney Island juvenile 1996; W97, winter 1997; SP97, spring 1997.

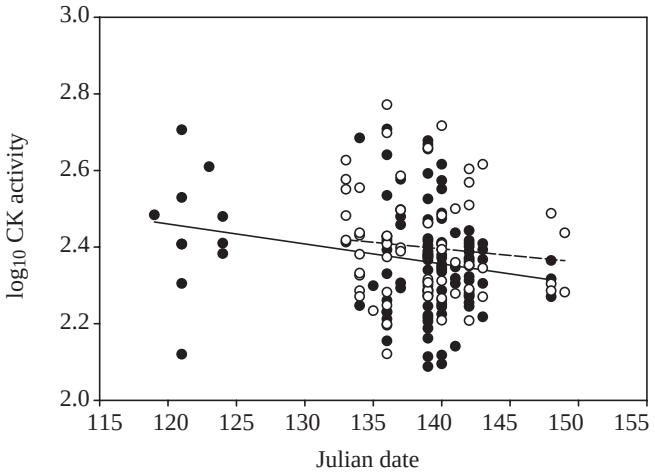

Fig. 3. The relationship between Julian date and $\log_{10}$-transformed plasma creatine kinase activity of bar-tailed godwits captured during a migratory stopover in the Wadden Sea. Closed circles, solid line, 'early arrivals'; open circles, dotted line, 'late arrivals'. Statistical analyses were performed on these data controlling for bleed-time, but the visual presentation of residuals would be nearly identical.

migratory stage and age combination ($P>0.10$), nor did age have any effect in wintering or pre-migratory birds ($P>0.27$); however, in fall at Boundary Bay, juvenile migrants had significantly higher plasma CK activities than adults ($P=0.04$). We also tested for an age difference with plasma collected in fall 1995 at Boundary Bay, and although all samples were stored for 9 months at −20 °C prior to analysis, juveniles again had higher plasma CK activities than adults ($P=0.02$). Plasma CK activity varied among migratory stages ($F=5.0_{1,275}$, $P=0.0001$; Fig. 2) with no stage-by-mass interaction ($P=0.49$). In 1996, pre-migrants had lower plasma CK activity than fall adult migrants ($P=0.01$), and tended to be lower than spring migrants ($P=0.03$). In 1997, wintering birds had lower plasma CK activity than spring migrants ($P=0.003$). When considered together, non-migrants had significantly lower plasma CK activity than all migrants combined (linear contrast $P=0.0001$). There was no difference in plasma CK activity between juveniles stopping at Sidney Island and Boundary Bay ($P=0.08$), or between spring and fall migrant adults ($P=0.69$).

Bar-tailed godwits

Plasma CK activity was negatively correlated with sample date in both years for this synchronous migrant (1997: $r=-0.14$, $P=0.045$; 1998: $r=-0.31$, $P=0.035$). Data from 1997 and 1998 were combined since we could detect no difference in CK activity between years ($P=0.87$), nor any interactions between year and bleed-time, body mass or date ($0.09<P<0.39$). Plasma CK activity did not differ between sexes, controlling for bleed-time and mass ($P=0.31$). Blood samples were obtained very quickly from godwits (median 2 min), yet a nearly significant positive effect of bleed-time on CK activity was still apparent ($F=3.0_{1,175}$, $r^2=0.02$, $P=0.09$). Controlling for bleed-time, there was no relationship between CK activity and body mass ($F=2.4_{1,174}$, $P=0.13$). Godwits captured at Castricum and birds showing evidence of body moult at Texel were classed as 'early arrivals', while birds not moulting at Texel were classed as 'late arrivals'. Controlling for bleed-time and arrival status, plasma CK activity declined with date ($F=4.4_{1,173}$, $P=0.018$). There was no interaction between date and arrival status ($P=0.85$), and taking into account the effect of date, late arrivals had higher plasma CK activity than early arrivals ($F=3.1_{1,173}$, $P=0.04$, Fig. 3).

Discussion

We found evidence of flight-induced muscle damage during migration in both western sandpipers and bar-tailed godwits, using a different investigative approach for each species. Our results indicate that, in addition to replenishing fuel stores (fat and protein), birds at migratory stopovers undergo some degree of repair to damaged flight muscles. Migrating western sandpipers had higher plasma CK activity than non-migrants in each year of study. In bar-tailed godwits, plasma CK activity was highest soon after arrival at the Wadden Sea and declined as birds prepared for the next leg of their journey. Also in agreement with our predictions, late-arriving godwits had higher mean plasma CK activity on a given day than birds that had arrived earlier. Late-arriving godwits also have lower body mass and reduced hematocrit (Piersma et al., 1996). It now seems apparent that behind-schedule godwits also may have less time to repair flight muscles before leaving the Wadden Sea for the breeding grounds.

Adult western sandpipers migrating in the fall are thought to make an over-water flight from western Alaska to British Columbia (2500–3000 km orthodrome), but they did not have

a greater index of muscle damage than spring migrants, which make short flights along a coastal route (Iverson et al., 1996; Butler et al., 1996). In contrast, body composition analysis indicated that migrants were more likely to deposit lean mass (including flight muscle) in the fall than in spring (Guglielmo, 1999), illustrating that utilization of muscle protein for energy is fundamentally different from muscle damage. Similarly, in herring gulls *Larus argentatus*, plasma CK, lactate dehydrogenase (LDH) and aspartate transaminase (AST) activities were unaffected by fasting, but increased on handling (Totzke et al., 1999). Hence, protein can be mobilized from the pectoralis without increasing the susceptibility to muscle damage and its associated functional impairment.

Migration may be especially challenging for young birds undertaking their first journey. There is good evidence that juvenile birds differ behaviourally from adults in ways that could reduce migration performance (e.g. inefficient foraging ability, low social status; Groves, 1978; Burger and Gochfeld, 1986; Hockey et al., 1998), but it is less clear if physiological factors are important. In the Arctic, shorebird chicks must grow quickly, and the pectoralis muscles grow most rapidly just prior to fledging (Hohtola and Visser, 1998). High growth rate is thought to be incompatible with the development of functional maturity of skeletal muscle (Ricklefs et al., 1998), and this could be particularly important for physiological systems that require training to achieve maximum performance (e.g. Pelsers et al., 1999). Like adults, juvenile western sandpipers must make an over-water flight to British Columbia as one of their first migratory flights. Juveniles at fall stopover (1995 and 1996) did not differ from adults in structural size, body mass, fat load, pectoralis muscle mass or pectoralis water fraction (an index of functional maturity; Guglielmo, 1999; C. G. Guglielmo, unpublished data). However, elevated plasma CK activity in juveniles in both years suggests that their relatively untrained state may put them at greater risk of muscle damage (and migration failure) than adults. The limited data available indicate that muscles of other young Arctic birds are biochemically mature prior to migration (Bishop et al., 1995; Pelsers et al., 1999; Olson et al., 1998). However, even after migration had begun, juvenile western sandpipers had lower muscle fatty acid binding protein levels than adults (Guglielmo, 1999), and perhaps fatty acid overloading could have exacerbated muscle damage in juveniles (Ockner, 1990). There remains much to be learned about the behavioural and physiological mechanisms affecting migration success in young birds, and how processes like learning and training (use/disuse) act as constraints on performance.

Flight-induced muscle damage has been studied in only a few other bird species. George and coworkers (George et al., 1987) examined flight muscle ultrastructure in Canada geese throughout the annual cycle, and found that after spring migration geese had visible evidence of extensive muscle damage, such as Z-line streaming and the presence of lipofuscin bodies. No evidence of damage was found in fall migrants, suggesting that in this case the stressfulness of the migration was an important determinant of muscle damage. Plasma CK levels were unchanged immediately after 1.5 h of flight in homing pigeons *Columba livia*, but this level of exercise may have been insufficient to induce muscle damage and plasma CK activity was not monitored in the hours or days following return (George and John, 1993). Plasma CK and LDH activities increased in response to flight training during rehabilitation of injured red-tailed hawks *Buteo jamaicensis* (Knuth and Chaplin, 1994). Bairlein and Totzke (Bairlein and Totzke, 1992) found a tendency for plasma CK, LDH and AST activities to be elevated during migration in five small passerine species, but did not control for the effect of capture, which we found to be substantial. In their study bleed-times of non-migrants (30 min) were much longer than of migrants (5 min), so the relative increases in plasma enzymes during migration were likely to be much greater than originally reported. Higher plasma enzyme levels in migrants were attributed to catabolism of muscle for fuel, but we suggest instead that these data indicate significant muscle damage in trans-Saharan migrant passerines.

Can muscle damage influence bird migration?

Interpretation of the functional significance of muscle damage for the behaviour and ecology of sandpipers and godwits is somewhat limited. Plasma CK activity is a useful indicator of muscle injury, but associating a given amount of increased CK activity quantitatively with a decrease in muscle function is difficult. Some authors have attempted to express CK activities as damaged muscle equivalents (Apple and Rhodes, 1988; Janssen et al., 1989). Others have argued that plasma CK activity does not relate to muscle damage quantitatively (Komulainen et al., 1995; Margaritis et al., 1999) or qualitatively (Komulainen et al., 1995). Nevertheless, even when ‘better’ plasma markers of muscle damage, such as myosin heavy chain, were used, their fluctuations were parallelled by changes in plasma CK activity (e.g. Koller et al., 1998). By modelling the kinetics of CK release and turnover, Volfinger et al. (Volfinger et al., 1994) suggested that a 100-fold elevation of plasma CK activity would be required to indicate significant myolysis in horses *Equus caballus*. Studies of humans and small mammals generally show significant changes in function (stiffness, soreness, edema, loss of strength) resulting from treatments that elevate plasma CK activity at least five- to 100-fold (Schwane et al., 1983; Clarkson et al., 1992; Nosaka et al., 1992, Balnave and Thompson, 1993; Rodenberg et al., 1993). A similar scale by which to judge muscle damage in birds is not available, but if this range (derived from mammals of a variety of body sizes) is taken as a benchmark, the increases in plasma CK activity that we found (1.2–2-fold) indicate that only moderate muscle damage occurred in migrant shorebirds.

Perhaps it should not be surprising to find low levels of muscle damage in shorebirds captured at migratory stopovers. First, it is possible that individuals with substantial muscle damage were not detected because they disappeared rapidly from the population (i.e. died or stopped migrating). Second, the Darwinian fitness costs of severe muscle damage could be

quite high, leading to strong selection for birds to manage flight distances and conditions to minimize damage. For example, muscle strength and mobility can decrease by half following damage (Clarkson et al., 1992), which would decrease flight performance and increase predation risk. Injured birds might need to devote more time to vigilance behaviour, or restrict their activities to the safest habitats. While damage can occur rapidly, the repair process can be prolonged (Clarkson et al., 1992; Clarkson and Sayers, 1999), and the best strategy for migrants may be to avoid muscle injuries in the first place. Third, the flight muscles could be superbly adapted to high intensity exercise, and possess morphological, physiological and biochemical mechanisms to prevent damage (e.g. antioxidants). Indeed this explanation seems likely, given that the exercise performance of migrants such as the bar-tailed godwit is so extreme, relative to activities known to cause significant muscle damage in other model systems (e.g. human marathon runners; Koller et al., 1998).

Even moderate muscle damage associated with migration could have an important influence on bird behaviour and ecology by impairing immune responses to pathogens. It is well known that intense repetitive exercise can cause immunosuppression (Fitzgerald, 1991; Nieman 1994). The repair of chronic, low-level muscle damage may be a contributing factor by constantly taxing the immune system (Shephard and Shek, 1998). Long-distance migrant shorebirds are high-performance exercise machines that can expend up to half of the annual energy budget during migration (Drent and Piersma, 1990). Piersma (1997) proposed that among shorebird species there is a physiological trade-off between migration distance (i.e. exercise performance) and pathogen resistance (i.e. immune function), which limits the longest distance migrants to relatively parasite-free locales (e.g. high arctic tundra and marine intertidal). As one mechanism for the trade-off, he suggested that the repair of muscle damaged by migratory flight impairs the immune defence against pathogens (Piersma, 1997). The detection of muscle damage in bar-tailed godwits and western sandpipers during migration provides partial evidence for these ideas, but more detailed studies will be required to fully test the hypothesis of a trade-off between migration distance and pathogen resistance, and its evolutionary consequences.

A major impetus for our study was a desire to understand how the physiological side effects of migratory flight can feedback to reduce subsequent performance at stopover, particularly by influencing settling time. Recent studies demonstrate that digestive system size is reduced by flight and fasting to such an extent that birds cannot fatten maximally at arrival (Klaassen and Biebach, 1994; Hume and Biebach, 1996; Klaassen et al., 1997; Biebach, 1998; Piersma and Gill, 1998; Karasov and Pinshow, 1998; Karasov and Pinshow, 2000; Lindström et al., 1999), and while our results are difficult to interpret functionally, they suggest that muscle damage is also a factor. Migration optimization models indicate that settling time can strongly influence optimal stopover duration and departure fuel load, but settling time is considered to be constant and unrelated to the duration or conditions of the previous flight (Alerstam and Lindström, 1990; Gudmundsson et al., 1991; Weber et al., 1994; Alerstam and Hedenström, 1998). If settling time is mainly a physiological phenomenon (not behavioural or ecological), its duration could be positively related to the length of the previous flight (i.e. longer flights entail longer fasting and more exercise). The implications of such a dependence of settling time on flight duration for the optimization of flight distances, fuel loads and stopover times has not been explored, but it seems to be a fertile subject for future theoretical and empirical study.

Capture stress

By far the highest plasma CK activities we measured were associated with capture stress. Plasma CK activity increased rapidly following capture in a mist net even though birds were removed as quickly as possible. 1–2 h after capture, plasma CK activity in western sandpipers was often in the range of 2000–5000 units l^{-1}, a tenfold increase from capture (C. G. Guglielmo, unpublished data). Plasma CK and AST activities have been used to assess the risks of capture myopathy in mallards *Anas platyrhynchos* (Bollinger et al., 1989; Dabbert and Powell, 1993). Bollinger et al. found that muscle damage varied among different capture methods, and was highest in enveloping type rocket nets. Dabbert and Powell reported that entanglement-type rocket nets caused more muscle damage than enveloping-type nets, and concluded that muscle damage could be minimized by reducing the amount and duration of physical struggling due to entanglement or physical handling by humans. Mist nets are generally safe for bird study (Gaunt and Oring, 1997). Our data indicate that mist net capture can cause some degree of muscle damage, but its possible effects on flight performance and survival are unknown. We suggest that muscle damage may be minimized by (1) using a small net mesh to reduce entanglement, (2) rapid removal of birds from nets, (3) minimization of human handling and (4) elimination of handling techniques that encourage eccentric muscle contraction. For example, extension of wings or legs against the efforts of the bird should be discouraged. Once in place for measurement a limb should be held firmly, as isometric contractions induce less muscle injury (Armstrong et al., 1991).

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