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Contrast in adaptive mass gains: Eurasian golden plovers store fat before midwinter and protein before prebreeding flight

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Before predictable periods of high nutritional demand and little or no intake, vertebrates store fuel mainly composed of energy-dense lipids or energy-poor but protein-rich muscle tissue. Documenting contrasts in fuel composition and storage patterns within species, or even within individuals, would greatly help to elucidate the functional significance of the variety of storage strategies demonstrated in birds. We show here that the 40–50 g mass gain of 200 g in Eurasian golden plovers (*Pluvialis apricaria*) in autumn in The Netherlands consists of fat only, but that the similar gain in body mass in spring consists of proteinaceous tissue (pectoral and other skeletal muscle and possibly skin tissue). That the same golden plovers store energy in autumn and store protein in spring suggests that they face energy deficits in early winter and risk protein deficits in spring, especially perhaps after arrival on the breeding grounds in late April and early May. In autumn and winter their diet consists largely of protein-rich earthworms, but upon arrival on Low Arctic and montane tundras, golden plovers tend to eat berries which are rich in sugars but notably poor in proteins. We therefore propose that the build-up of proteinaceous tissue in spring reflects a strategic storage of a nutritional resource that is likely to be in short supply somewhat later in the year.

Keywords: frugivory; fuel; migration; phenotypic flexibility; storage strategies

1. INTRODUCTION

Over the past decade, it has become well established that the composition of energy stores strategically deposited by birds before periods of food scarcity and/or high energy demand rarely consists entirely of fat (McLandress & Raveling 1981; Piersma 1990; Lindström & Piersma 1993; Jehl 1997; Biebach 1998; Piersma *et al.* 1999). But the extent to which wet protein is stored varies greatly between species and situations (Lindström & Piersma 1993; Piersma 1998). This variability is not understood. The extent to which various organ systems show hypertrophy or atrophy during fuel storage is also variable. In this case reductions in the size of the digestive organ system before take-off appear associated with long migration flights over inhospitable terrain (Piersma 1998).

Contrasts in fuel composition and storage patterns within species, or even within individuals, would greatly help to elucidate the functional significance of the variety of storage strategies (Piersma & Lindström 1997; Piersma 1998). The doubling of their body mass by king penguins (*Aptenodytes patagonicus*) at sea before long fasts on land consists of 14% protein in penguins that are going to incubate and 29% protein in penguins that are beginning a protein-demanding moult of their pelage (Cherel 1995). Here we demonstrate an even more striking difference in the composition of a 40–50 g mass gain by 200 g Eurasian golden plovers (*Pluvialis apricaria*) during stopovers in The Netherlands in autumn (before they move on to wintering destinations in France, Spain and Portugal) and in spring (before they move on to the breeding grounds in Scandinavia and northwestern Russia), and provide a functional interpretation of this difference. Compositional changes are described in terms of fat and fat-free tissue (wet protein), and in terms of the size of various organs. As the population of golden plovers sampled in The Netherlands in autumn and spring is the same, and as body-mass changes are fairly synchronized between individuals (Jukema *et al.* 2001), series of sacrificed individuals are likely to yield good estimates of the changes occurring within an individual in the course of a nonbreeding season (Lindström & Piersma 1993; van der Meer & Piersma 1994).

2. METHODS

A total of 51 Eurasian golden plovers, inadvertently killed during daytime catches at grassland sites in the province of Friesland by using so-called 'wilsternets' (Jukema *et al.* 2001), were collected from 1988 to 1992. Body-mass changes are usually highly repeatable from year to year (Jukema & Piersma 1997). Birds were selected from two time windows when body mass increased at constant rates (Jukema *et al.* 2001): the periods from 12 October to 12 December 'autumn' ($n = 23$) and 1–16 April 'spring' ($n = 28$).

Within half a day after death the carcasses were stored in airtight plastic bags in a freezer at temperatures below −20 °C.

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Table 1. Average values of body mass, total fat mass, total fat-free mass and the fat-free dry masses of the total body and the different parts in autumn and spring, and the parameter values for the linear regressions on days from the start of the mass increase in autumn and spring of all these mass components.

(The slopes of the regressions that were significant at the 5% level are printed in bold along with the accompanying r^2 and p values. Total fat-free mass is calculated as body mass minus total fat mass.)

	autumn ($n = 23$)				spring ($n = 28$)			
body part	mean	r^2	p	slope	mean	r^2	p	slope
body mass	225.9	**0.38**	**0.002**	**0.62**	213.9	**0.41**	**<0.001**	**2.26**
total fat mass	43.4	**0.60**	**<0.001**	**0.61**	17.1	0.02	0.475	—
total fat-free mass	182.5	<0.01	0.910	—	196.8	**0.44**	**<0.001**	**2.00**
total fat-free dry mass	62.2	0.01	0.628	—	65.8	**0.45**	**<0.001**	**0.59**
skin	7.28	0.06	0.247	—	8.34	0.11	0.083	(0.13)
pectoral muscles	13.56	0.08	0.199	—	14.36	**0.19**	**0.022**	**0.11**
empty carcass	19.84	0.06	0.247	—	21.77	**0.24**	**0.008**	**0.26**
heart	0.86	0.15	0.066	(0.004)	0.99	0.01	0.588	—
lungs	0.84	**0.38**	**0.002**	**0.004**	0.87	0.03	0.424	—
leg muscles	2.59	<0.01	0.723	—	2.70	0.02	0.516	—
stomach	1.55	0.10	0.141	—	1.46	0.05	0.252	—
intestine	1.54	**0.33**	**0.004**	**−0.01**	1.37	**0.17**	**0.026**	**0.03**
liver	2.34	0.02	0.556	—	2.76	0.07	0.179	—
kidneys	0.72	0.05	0.286	—	0.79	<0.01	0.867	—
brain	0.27	<0.01	0.831	—	0.27	0.02	0.519	—

Specimens were thawed for a day at room temperature before dissection. The birds' ages were assessed as juvenile (first winter) ($n = 12$) or adult (older than one year) ($n = 33$) on the basis of feather characteristics (the presence of secondaries of various ages is diagnostic for adults; Jukema *et al.* 2001). In six cases age could not be determined with confidence. Note that Eurasian golden plovers start breeding when they are 1 year old and do not show age-related differences in seasonal mass changes (Jukema *et al.* 2001).

We measured maximum wing length (to the nearest mm), tarsus plus toe length (to the nearest mm), bill length (exposed culmen) and total head length (both to the nearest 0.1 mm). After plucking, the skin was opened with a cut along the keel and stripped off the rest of the carcass together with tibiotarsi and feet. Next, the pectoral muscles (the small musculus supracoracoideus and the large m. pectoralis) on both sides of the keel were removed. In all birds six measurements of the sternum and coracoid were taken: four of them followed the instructions of Piersma *et al.* (1984). In addition, the length of the sternum on the dorsal side and the distance between distal tip of the coracoid and dorsal end of the keel were measured with callipers to the nearest 0.01 mm (Jukema & Piersma 1992). Sex was determined by gonadal inspection. Thereafter, the remaining parts of the body (legs, stomach, intestine, heart, lungs, liver, kidneys) were excised until an 'empty carcass' remained. In the case of the legs, the muscle tissue was removed from the bones, the latter being included with the 'empty carcass', which thus contained most of the skeleton with some attached skeletal muscle.

All parts were dried separately to constant mass at 60 °C. The dried tissues were weighed to the nearest 0.1 g, packed in filter-paper and then fat extracted in a Soxhlet apparatus, using petroleum–ether (boiling point range of 40–60 °C) as the solvent. Fat extraction was considered complete when the solvent in the extraction vessel no longer showed any yellowish colour (i.e. indicating that fat was no longer in solution) for three successive fillings (usually after 2–4 h). The parts were dried to constant mass again, separated from the filter-paper and the remaining fat-free dry mass weighed. The mass of fat (non-polar lipids only) was estimated from mass loss during extraction.

A multivariate estimate of structural size (the first principle component based on a principal component analysis (PCA) of the four external and the six internal structural measurements) showed no differences in mean size between identifiable juveniles and adults (pooled-variance Student *t*-test, $p = 0.11$), or between males and females ($p = 0.92$). There was a minor but statistically significant difference between the autumn and spring group ($p = 0.03$; the birds from autumn were smallest) that must have been due to a sampling bias (birds passing The Netherlands in autumn and spring have been shown to belong to the same population; Jukema *et al.* 2001). In the absence of within-season variations in body mass (see below), values were not corrected for variation in structural size. Averages are presented with 1 s.d. Differences between averages were tested with pooled-variance Student *t*-tests, with a p value of 0.05 being interpreted as statistically significant. Statistics were performed using SYSTAT.

3. RESULTS

That the average body mass of golden plovers in autumn (225.9 ± 20.2 g) was 6% higher than in spring (213.9 ± 15.9 g) was due to the average amount of fat stored in autumn being 2.5 times as high as in spring (43.4 ± 14.9 g and 17.1 ± 8.3 g, respectively). Fat-free mass in autumn (182.5 ± 9.4 g) was actually 7% lower than in spring (196.8 ± 13.7 g). These seasonal differences between fat-free mass are due to significantly higher fat-free mass values in spring than in autumn (with 5–15%) of skin, pectoral muscles, empty carcass, heart and liver (table 1). Only dry feather mass (10.78 ± 1.24 g and 10.11 ± 0.70 g, respectively) and intestinal mass were significantly higher in autumn than in spring (with 7% and 11%, respectively). In neither season were we able to detect statistically significant differences between any of the mass values of juveniles and adults; only the smaller

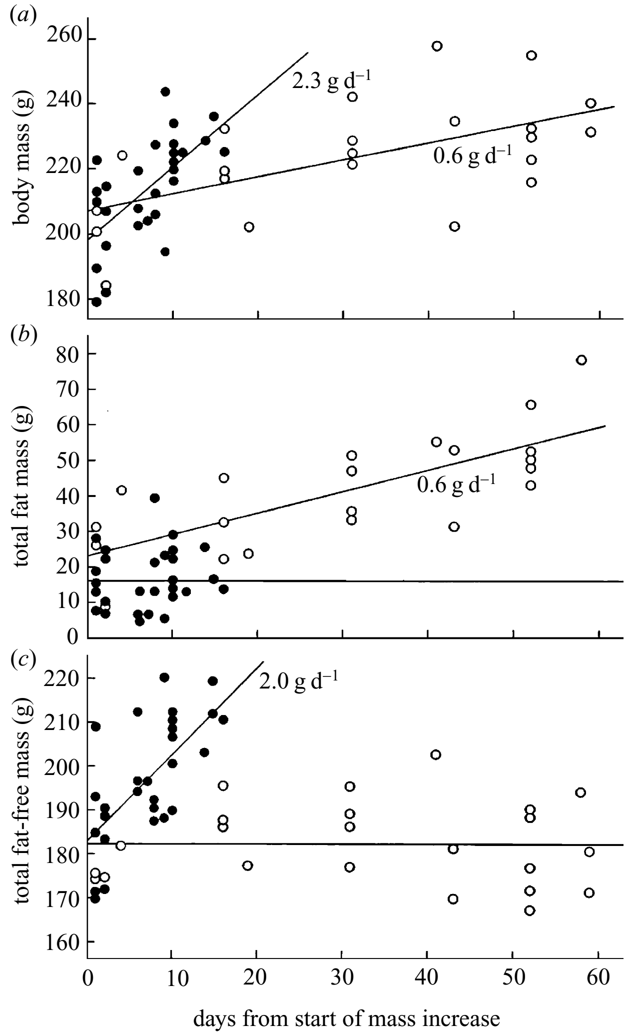

Figure 1. Increases in (*a*) body mass, (*b*) total fat mass and (*c*) total fat-free mass of Eurasian golden plovers on day from the start of the mass increases in autumn (white circles) and spring (black circles). Only the significant slopes (in g d^{-1}) are presented with figures. See table 1 for additional statistics.

fat-free masses of juveniles than of adults in autumn approached significance ($p = 0.052$)

In autumn, the body-mass increase of the sampled birds (table 1 and figure 1) was similar to the body-mass increase shown by the population (sample: 0.6 g d^{-1}; population ($n = 534$): 0.83 g d^{-1} from 12 October to 12 December 1988–2000). In spring, the body-mass increase of the sample was higher than that of the population (sample: 2.3 g d^{-1}; population ($n = 277$): 1.04 g d^{-1} from 1–16 April 1988–2000), which may be due to the higher degrees of asynchrony of spring fuelling if a larger number of years and more individuals are incorporated (Zwarts *et al.* 1990). Neither in autumn nor in spring was there any evidence for body size changes over time (the first principle component of the PCA of four external and six internal structural measurements did not correlate with date; p values of 0.144 in autumn and 0.599 in spring).

An increase over time in body mass was apparent in both the autumn and the spring samples, but total fat mass increased only in the course of autumn, whereas total fat-free mass increased only in spring (table 1 and figure 1). Indeed, the entire mass increase in autumn can be accounted for by an increase in stored fat. The slight but significant decrease of the fat-free mass of the intestinal tract was compensated for by a similarly small increase in the size of the lungs (table 1).

In spring, most of the 2.26 g d^{-1} rate of increase in body mass can be accounted for by the daily gain in fat-free mass (2.00 g d^{-1}; table 1). This increase translates into a daily gain of 0.59 g fat-free dry mass that appears composed of increases in the empty carcass (0.26 g d^{-1}), the pectoral muscles (0.11 g d^{-1}) and the intestine (0.03 g d^{-1}). If we add up these body parts, 0.40 g d^{-1} of the 0.59 g d^{-1} increase in fat-free mass is explained. However, if the (marginally significant) 0.13 g d^{-1} increase in skin mass is also included (the total is then 0.53 g d^{-1}), most of the daily increase in proteinaceous tissue is accounted for.

4. DISCUSSION

Just as in Eurasian golden plovers, the peak in winter mass in two other large wader species wintering at temperate latitudes, the bar-tailed godwit (*Limosa lapponica*) (Evans & Smith 1975) and the oystercatcher (*Haematopus ostralegus*) (Zwarts *et al.* 1996), consisted almost entirely of additionally stored fat. A preliminary and crude analysis of the body composition of golden plovers (Piersma 1998) suggested fat contributes 35% rather than 0% to their body-mass gain in spring, but nevertheless it was the lowest proportion of fat stored among the four wader species examined. In their review of the relative contributions of the fat and non-fat components to increases in body mass before migration flights, Lindström & Piersma (1993) found no species where the fraction of fat was lower than 40%. Therefore, that golden plovers only stored fat in autumn is in line with previous findings. That they appeared to store no additional fat in spring is somewhat exceptional.

Golden plovers staging in The Netherlands in April are 500–3000 km away from their breeding grounds (Byrkjedal & Thompson 1998; Jukema *et al.* 2001). This distance is probably covered in a series of short flights using various stopover sites, e.g. in northern Germany and southern Scandinavia. A store of fat maintained at 15–20 g is enough to fuel such successive flights. However, that golden plovers store protein in spring and energy in autumn suggests that they are facing protein deficits in spring, perhaps especially after arrival on the breeding grounds in late April and early May. Likewise they must be facing energy deficits in early winter.

Their autumn and winter diet consists largely of protein-rich earthworms (Lumbricidae). The lack of protein storage in autumn can easily be explained by the fact that one or more earthworms per day may cover the protein requirement of golden plovers. What they need in such times is energy. Upon arrival on Low Arctic and montane tundras golden plovers tend to rely heavily on berries (such as crowberries, *Empetrum* spp.), which represent the first edible items to emerge from under the melting snow (Byrkjedal & Thompson 1998). Berries tend to be rich in sugars but notably poor in proteins (Klasing 1998). For example, crowberries contain only 0.7 mg N g^{-1} fresh mass, but as much as 16.5 mg lipid and 19.4 mg carbo-

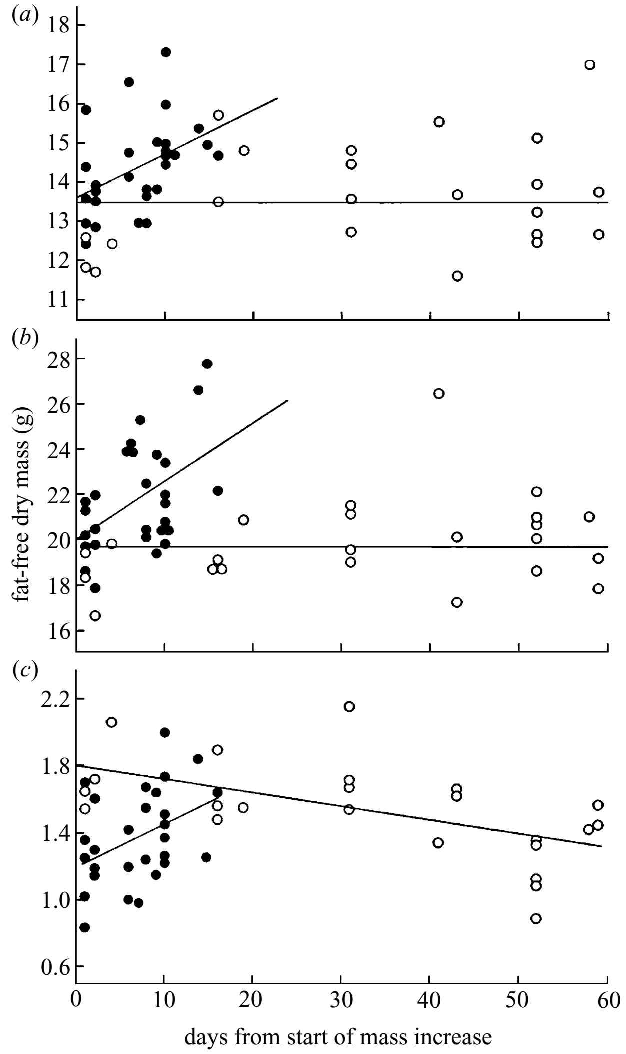

Figure 2. Increases in the fat-free dry masses of (*a*) the pectoral muscles, (*b*) the empty carcass and (*c*) the total empty intestinal tract of Eurasian golden plovers on day from the start of the mass increases in autumn (white circles) and spring (black circles). See table 1 for additional statistics.

hydrate (Eriksson & Ehrlén 1991). We therefore propose that the build-up of proteinaceous tissue in spring reflects a strategic storage of a nutritional resource that is likely to be in short supply later in the year.

The lack of changes in the size of the pectoral muscles with increasing mass in autumn in golden plovers (figure 2) differs from the findings based on non-invasive ultrasonographic techniques that the pectoral muscles of red knots (*Calidris canutus*) track changes in body mass (Dietz *et al.* 1999; Lindström *et al.* 2000). Equally intriguing is the decrease in intestinal mass in the course of autumn fattening, which apparently is followed later by a rapid build-up in spring (figure 2). Without detailed knowledge on dietary changes in the course of the winter, we cannot tell whether these changes reflect seasonal changes in diet (cf. Klasing 1998). That the protein deposits left on the otherwise cleaned, empty carcass, and perhaps also the skin, are used as protein stores in spring, echoes the finding that red knots lose 20% of the fat-free (and feather-free) skin mass over the summer in the High Arctic (Piersma *et al.* 1999), and that in great knots (*Calidris tenuirostris*) the fat- and feather-free mass of the skin decreases by a quarter during a 5400 km non-stop northward flight (Battley *et al.* 2000, 2001).

A close relative of the Eurasian golden plover, the Pacific golden plover (*Pluvialis fulva*), was the first long-distance migrating wader species for which body compositional data bearing on wintering and migration became available (Johnston & McFarlane 1967). That the fat-free dry masses of birds ready to fly north in April ($n = 11$) were 16% higher than those of birds in December ($n = 12$) received no comment then. In a much more comprehensive study, Johnson *et al.* (1989) showed increases in fat-free dry mass between the autumn and spring of 23% in juveniles and 13% in adults. Their conclusions for Pacific golden plovers wintering in the Hawaiian archipelago and breeding in Alaska are thus generally consistent with ours.

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