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Seasonal variation in energy expenditure and body composition in captive White Storks (*Ciconia ciconia*)

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ABSTRACT

North Western European populations of White Storks (*Ciconia ciconia*) appear to have been saved from extinction by settling, i.e. stopping migration. Settled storks exposed to winter conditions must cope with periods of potentially high energy demands that would otherwise be avoided by the migration process. Doubly labeled water (DLW) was therefore used to examine the seasonal variation (summer vs winter) in daily energy expenditure (DEE) and the body composition of adult and immature storks of both sexes. Male White Storks showed a higher DEE over the winter period than in summer compared with females; in particular, immature males exhibited greater energy expenditure in winter than adult males. Thus, the DEE did not significantly differ between summer and winter (except for immature males), reflecting an absence of thermoregulation cost in winter. For both age classes, total body mass increased in winter, which was mainly due to an increase in fat mass. Adult storks were 5% heavier than immature storks. The sexes differed in body mass, with males weighing significantly more than females by 11%. Mean LBM (lean body mass) was 8.5% higher in adults than in immatures, and was 11.5% higher in males compared with females. Between their first and second summers, immatures accumulated a lean body mass to finally reach the same values as adults, indicating a phase of muscle development. The mean fat mass of the storks did not differ between age classes or between sexes. Based on physiological parameters, this study shows that settled White Storks are able to cope with mild winter periods when they are artificially provided with food. In a view to preserve favourable habitats for this species, it is therefore necessary to decide on a plan of action for breeding areas.

1. Introduction

Winter in temperate regions is characterized by low ambient temperatures and food scarcity. However, some species of birds are able to inhabit northern latitudes and they have to develop strategies in order to cope with harsh climatic conditions (King, 1974; King and Murphy, 1971). They show metabolic adjustments (a capacity of increased heat production), a greater insulation and some modifications of nutritional and behavioural characteristics (Dawson and Carey, 1976). Other species of birds breeding in these north temperate regions migrate before winter to southern latitudes with more abundant food and undemanding climatic conditions. Daily energy expenditure, one of the physiological variables likely to be affected by environmental fluctuations, is useful for evaluating the adaptation of birds to cold environments. In comparison with small birds, large birds (>200 g), show reduced heat loss in relation to their large size; they also have a lower basal metabolic rate (Calder and King, 1974). Although larger species certainly require more food, they have a relatively higher fasting capacity due to a lower mass-specific basal metabolic rate (Calder and King, 1974). Moreover, although some studies fail to show consistent patterns of seasonal variation in metabolic rate (Bech, 1980; Sherfy and Pekins, 1994; Swanson and Weinach, 1997), other works found a lower basal metabolic rate during winter compared with summer for several large species of birds weighing over 200 g (Weathers and Caccamise, 1978).

The White Stork (*Ciconia ciconia*) breeds in Europe and migrates during fall to wintering areas, i.e. South Western Europe, the Middle East and Northwest Africa. The population of White Storks has greatly declined in the Alsace region (North-East of France) since 1960. This decline was partly attributed to both an increased mortality during migration and to limited food availability in African wintering areas due to Sahelian droughts (Dallinga and Schoenmakers, 1987; Kanyamibwa, et al., 1990). As part of the initiative to protect this species, young birds were maintained in captivity for a three year period (Schierer, 1986). After release, some of these individuals were observed to have ceased their migratory behaviour, and remained in the Alsace over winter. In this context, the settled storks exposed to winter conditions must cope with periods of potentially high energy requirements which are unnecessary in usual migratory behaviour. The migratory pattern and population dynamics of this settled species have

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raised considerable interest (Barbraud, et al., 1999; Kanyamibwa, et al., 1990), and some studies have been carried out to investigate energetic aspects of fasting in this species in a cold environment (Mata, et al., 2001).

The aim of this study was to evaluate the effects the winter season has on energy requirements and body reserves using doubly labeled water taken from settled captive White Storks fed *ad libitum* and housed outdoors all year round. For this, we compared daily energy expenditure and body reserves according to age (immature and adult), sex, and season (winter and summer) in captive White Storks fed *ad libitum*. We predicted that the daily energy expenditure of birds should be higher in winter than in summer as a result of higher thermoregulatory costs. Because the White Stork is a protected species, we used the doubly labeled water method (Lifson and McClintock, 1966; Speakman, 1997), a non-invasive technique, to determine the daily energy expenditure and to evaluate the body composition of the same individuals during different phases of the annual cycle.

2. Materials and methods

2.1. Birds

White Storks (*C. ciconia*) were kept in a large outdoor aviary (110 m^2 stocking 20 birds) exposed continuously to natural temperatures and photoperiods. We studied 7 adults (3 females and 4 males between 8 and 25 years old) and 6 immatures (3 males and 3 females, all 3 years old). They were followed over three consecutive periods: summer I (July 1997), winter (January 1998) and summer II (July 1998). During the periods of study, daily air temperatures ranged from 14 °C to 25 °C (mean 20 °C) in summer I, from 8 °C to −4 °C (mean 3 °C) in winter and from 13 °C to 25 °C (mean 19 °C) in summer II (D. Bonn, correspondent of France Météo, Strasbourg). Birds were fed with dead rooster chicks (*Gallus gallus domesticus*) provided *ad libitum*. The study was carried out in Strasbourg (France).

2.2. Doubly labeled water (DLW) experiments

Energy expenditure was measured using the doubly labeled water method (DLW) (Lifson and McClintock, 1966). The birds were food- and water-deprived 2 h before each experiment. They were weighed accurately (±5 g) and blood samples were taken to determine the oxygen-18 (^{18}O) and deuterium (^2H) background values of body water. The DLW consisted of highly enriched water made up of $H_2^{18}O$ (64%) and ^2H_2O (32%). Birds were injected in the pectoral muscle. The amount of dose injected (about 0.4 g per kg of body mass) was determined to the nearest 0.1 mg by weighing the syringe before and after injection. Previous tests indicated that the isotope ratios attained their highest values within 1 h after injection. Birds were left undisturbed for 31/2 h after injection to reach isotope equilibrium with body water (Mata, et al., 2001), and the initial blood sample was taken from the wing brachial vein to determine the initial isotope enrichments. Three other blood samples were collected 1, 3 and 6 days after injection to measure the isotope elimination rates. At each sampling, two to three 100 μL capillaries were filled with approximately 80–90 μL of blood. They were flame-sealed and stored at 5 °C until analysis.

2.3. Isotope analysis

For each sample, $^{18}O/^{16}O$ and $^2H/H$ isotope ratios were determined in our facility with a VG Isotopic Analyzer Mass Spectrometer. Water was extracted from blood samples by vacuum distillation (Wong, et al., 1987). We determined ^{18}O and ^2H enrichments with CO_2 equilibration and zinc reduction methods, respectively (Wong, et al., 1987). The $^{18}O/^{16}O$ ratio was measured after equilibrating 15 μL of the water sample with a fixed volume of CO_2 (0.04 mM) of known enrichment for 48 h at 25 °C in a thermostatic-controlled cabinet (Aqualytic), to allow the exchange of ^{18}O atoms between the body water sample and the CO_2. After equilibration, the CO_2 was separated from the water by freezing it to −80 °C using a dry acetone bath and taking the CO_2 with liquid nitrogen trap. The $^{18}O/^{16}O$ enrichment for the water could be calculated from the enrichment of CO_2 and from the amount of water and CO_2 involved in the equilibration process (Wong, et al., 1987). For deuterium analysis, an aliquot of 3 μL of water was reduced with 100 mg of zinc as a catalyzer (provided by the Geological Department, Indiana University, USA) in a hot-furnace (Dri-Block DB-4, Techne) at 485 °C for 30 min. For each background, initial and following samples the $^{18}O/^{16}O$ and $^2H/H$ isotope ratios were measured in triplicate. All sets of isotope measurements were calibrated using working reference solutions previously calibrated according to the International Atomic Energy Agency standards.

2.4. Isotope dilution spaces

For each bird the initial ^{18}O and ^2H dilution spaces (*g*, N_O and N_D, respectively) were calculated on the basis of the principle of isotope dilution. Both dilution spaces were calculated by the extrapolation or intercept to zero time, where the volume is calculated from the initial dilution obtained by extrapolating lines back to zero (Coward, 1990). Dilution spaces were calculated as explained in Mata et al. (2001):

$$N_O = \alpha_O \times \left(\frac{m_{inj}}{\Delta R_O}\right) \text{ and } N_D = \alpha_D \times \left(\frac{m_{inj}}{\Delta R_D}\right) \tag{1}$$

where m_{inj} (in g) is the amount of injected dose. ΔR_O and ΔR_D are the initial ^{18}O and ^2H enrichments of body water. The α_O and α_D coefficients characterize the ^{18}O and ^2H concentrations of the injected solution. These were determined by measuring the $^{18}O/^{16}O$ and $^2H/H$ enrichments of distilled water resulting from the addition of *m* grams of the injected solution to *M* grams of water. The backgrounds of $^{18}O/^{16}O$ and $^2H/H$ ratios were 2000.0 ± 0.7 ppm and 151.8 ± 0.5 ppm, respectively.

2.5. Rate of CO_2 production and daily energy expenditure (DEE)

For each bird, the rate of CO_2 production (rCO_2, L d^{-1}) was calculated from total body water (*N*) and the ^{18}O and ^2H turnover rates using equation 7.17 of Speakman (1997):

$$rCO_2 = (N/2.078) \times (k_O - k_D) - 0.0062 \times N. \tag{2}$$

This equation assumes that 25% of the water lost from the body is lost as vapour, and corrects for fractionation effects in the evaporated water. The conversion of CO_2 to heat production depends on the composition of catabolised substrates. To convert units of CO_2 production to energy expenditure, we used the currently reported value of 25.7 kJ L^{-1} CO_2 corresponding to a proteinaceous food resource (Ricklefs, 1974).

2.6. Body composition

The most direct and accurate method for determining body reserves is carcass lyophilisation (Robbins, 1993). However, this procedure is forbidden when dealing with an endangered and legally protected species such as the White Stork. To estimate the body composition of each stork, total body water mass (TBW) was determined using the method of isotope dilution of ^{18}O. TBW was shown to be proportional (Pace and Rathbun, 1945) to the lean body mass (LBM). Then, from a data set on the body composition of accidentally killed White Storks (Michard-Picamelot, 1999), we estimated the ratio of TBW to LBM and found it to be 0.739. We used the following relationships (Pace and Rathbun, 1945) to determine the proportions of LBM and fat mass (FM) of our experimental storks:

$$LBM\% = TBW\% / 0.739 \tag{3}$$

$$FM\% = 100 - LBM\%. \tag{4}$$

Table 1
Results of repeated-measures ANOVA for the five variables.

		Body mass (g)		Fat mass (g)		Lean body mass (g)		Adiposity (%)		DEE (kJ d^{-1})	
Model term	d.f.	MS	F	MS	F	MS	F	MS	F	MS	F
Age	1	310,225	9.54*	4959	0.12	477,810	97.76*	0.92	0.11	21,424	0.45
Sex	1	1,697,811	52.22*	123,814	3.01	886,675	181.79*	0.01	0.001	297,242	6.22*
Age × sex	1	27,578	0.85	6841	0.17	8621	1.77	0.545	0.07	5694	0.119
Error	9	32,513	–	41,082	–	4877	–	8.30	–	47,807	–
Season	2	992,241	115.53*	806,281	188.46*	90,189	20.89*	161.87	178.23*	80,881	17.54*
Season × age	2	142,381	16.58*	193,774	45.29*	59,278	13.73*	73.34	80.76*	4851	1.05
Season × sex	2	365	0.04	1058	0.25	1275	0.30	0.22	0.24	8675	1.88
Season × age × sex	2	894	0.10	94	0.02	1249	0.29	0.17	0.19	19,523	4.23*
Error	18	8588	–	4278	–	4317	–	0.91	–	4611	–

*$p < 0.05$.

2.7. Statistical analysis

The normality and the equality of variance were checked before carrying out a Bartlett's test. Data expressed as percentages were transformed using the arcsine transformation. Body composition and energy expenditure variables were modelled by using repeated-measures ANOVA. There were two “between” factors, age and sex and one “within” factor, season. Since statistical comparisons performed on mass-specific metabolism are unreliable (Hayes, 2001; Packard and Boardman, 1999), seasonal, age and sex variations of daily energy expenditure were tested by using an ANCOVA with repeated measures. Subsequent pairwise comparisons were made using the Student–Newman–Keuls (SNK) method (all statistical tests were performed using STATISTICA® version 5.1). Means are presented with standard errors and results were significant at $p < 0.05$.

3. Results

3.1. Energy expenditure

Because the season × age × sex interaction was significant (Table 1), we performed two-way repeated ANOVA (season × age) separately for males and for females. The main effect of season on males was significant ($F_{2,10} = 16.64$; $p < 0.001$). Immature males experienced a significantly greater increase of energy expenditure (in kJ/day, Fig. 1) during winter than that seen in adult males (season × age interaction, $F_{2,10} = 4.91$; $p < 0.05$). Females did not show significant seasonal variation in energy expenditure. We found similar results when we included body mass as a covariate in a repeated-measures analysis of covariance for each sex (interaction age × season, $F_{2,8} = 5.90$; $p < 0.05$ for males and no significant interaction for females).

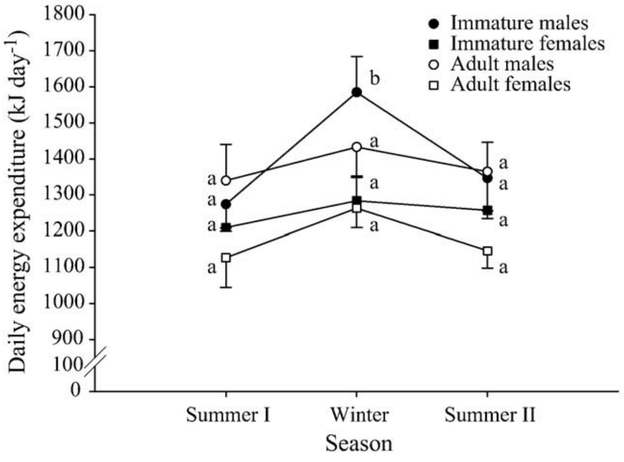

Fig. 1. Variation of daily energy expenditure according to age, sex and season. Mean ± standard error, those labeled with different letters are significantly different (ANOVA with repeated measures and multiple comparisons made using the SNK).

3.2. Body mass

There were significant differences in body mass between adult and immature storks, adults being 5% heavier compared with immatures (Table 1, Fig. 2). Furthermore, the sexes differed in body mass, the males weighing significantly more than females by 11% (Table 1; males: 3646 ± 40 g; females: 3225 ± 43 g). Both adults and immatures presented the same seasonal pattern of body mass change, characterized by a significant increase in winter followed by a decline in summer II (Table 1, Fig. 2A). However, body mass increase was higher in adults than in immatures (22 vs 9%, significant interaction season × age), meaning that adults were heavier than immatures in winter (Table 1).

3.3. Body composition

Mean LBM was 8.5% higher in adults than in immatures and it was 11.5% higher in males compared with females (Table 1). The season had a significant effect on LBM with a significant increase of this parameter in both age classes in winter. Adults accumulated more LBM than immatures in winter (significant interaction season × age). In summer II, lean body mass of immatures reached the same value as that seen in adults (Fig. 2B).

The mean fat mass of the storks did not differ between age classes or between sexes (Table 1). There were significant differences in fat mass through seasons and also a significant season × age interaction (Table 1, Fig. 2C). Thus, in summer I, immatures were significantly fatter than adults (Fig. 2C; adiposity of 29 vs 23%, Fig. 3B). However, their body composition varied noticeably during the fourth year of growth since, in summer II, their adiposity declined to a low level, even lower than that measured for adults (20 vs 24%). Since body mass in immatures was similar between the two consecutive summers, this marked decrease in adiposity during summer II reflected a decrease in fat mass, which was almost entirely replaced by an increase in lean mass (9%, Figs. 2B and 3, Table 1). In winter, fat mass (Fig. 2C) and adiposity, i.e., the amount of fat in proportion to body mass, increased significantly in both adults and immatures (Fig. 3). The increase in fat mass accounted for 60–70% of the increase in total body mass. Immatures, however, which had a higher adiposity than adults in summer I, accumulated much less body lipid (200 vs 430 g), so that adiposity in winter was similar between immatures and adults. In adults, LBM% and adiposity did not change markedly between the two summers.

4. Discussion

In this study we measured energy expenditure and body composition, using the doubly labeled water method, in captive White Storks fed *ad libitum* and housed outdoors all year round. White Storks present a seasonal DEE (daily energy expenditure) variation in males, this value

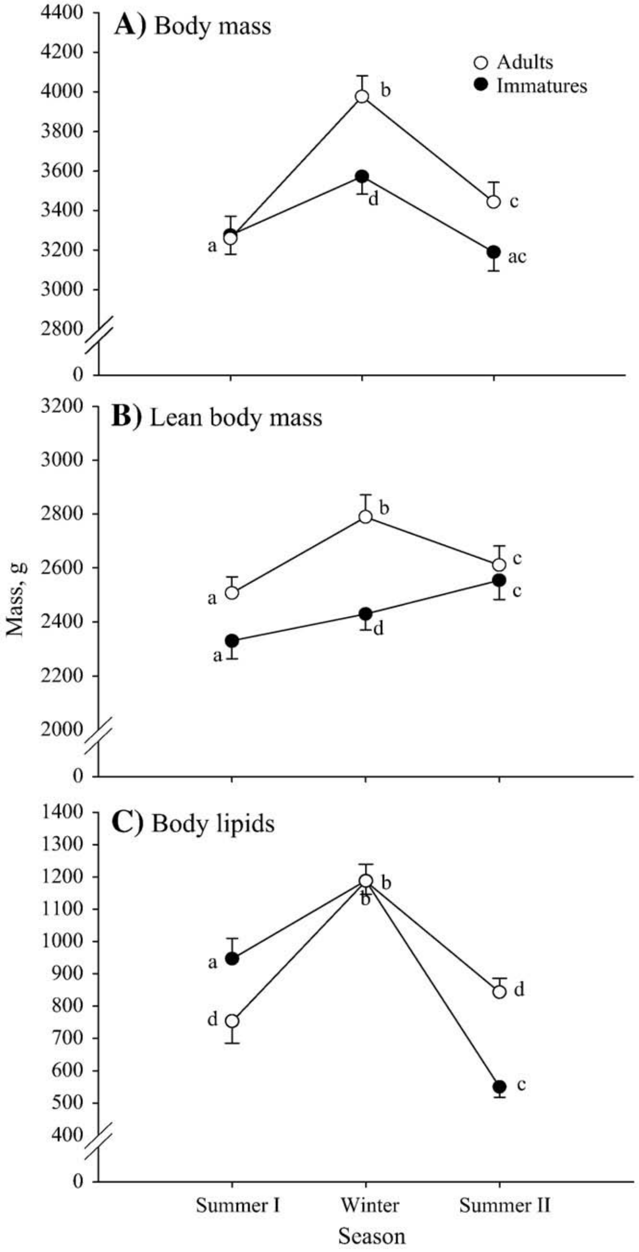

Fig. 2. Seasonal and age variation of body mass, lean body mass and fat mass of adult and immature white storks. Mean ± standard error, those labeled with different letters are significantly different (ANOVA with repeated measures and multiple comparisons made using the SNK).

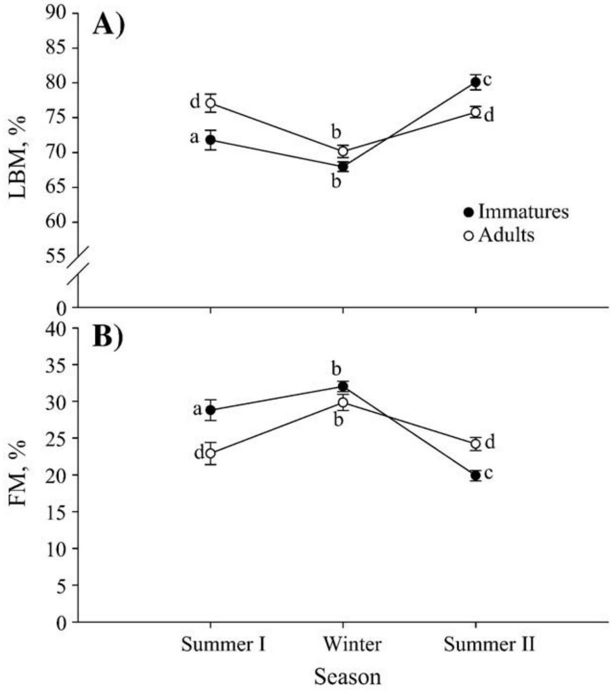

Fig. 3. Seasonal and age variations of lean body mass (A) and fat mass (B) percentages. Values are mean ± standard error, those labeled with different letters are significantly different (ANOVA with repeated measures and multiple comparisons made using the SNK).

being higher in immature males. Body mass also presents a seasonal variation principally reflected as an accretion of body fat.

4.1. Seasonal variations in daily energy expenditure

Male White Storks showed a higher DEE during winter than in summer, compared with females (Table 1). In particular, when compared within sex, immature males exhibited greater energy expenditure in winter than adult males whereas no differences were observed between immature and adult female storks. The higher and more variable DEE of immature males indicates that winter conditions have a greater impact on them. This result could be related to the accumulation of protein mass during this period (Fig. 2B), reflecting an active phase of muscle development as described in other growing species (Starck and Ricklefs, 1998). With the exception of immature males, DEE did not change significantly between summer I, winter and summer II, reflecting a constant thermoregulation cost. This could be explained by 1) the moderate winter temperatures, the difference between winter and summer temperatures in these experiments that did not exceed 17 °C; and 2) the high lipid content of the storks during winter, which ensures an efficient thermal insulation since more than 50% of fat mass is stored in subcutaneous tissues (Bairlein and Gwinner, 1994; Blem, 1976).

Energy expenditure in endotherms is constrained between upper and lower limits. Data on the basal metabolic rate (BMR) of White Storks are lacking. Hence, as a reference for the lower limit of energy expenditure we used the conventional allometric equation derived by McKechnie and Wolf (2004) for 126 avian species. From this relationship, the storks had a BMR of 670 kJ d^{-1} for a 3230 g stork in summer and 730 kJ d^{-1} for a 3700 g stork in winter. Expressed as a multiple of BMR, DEE was 1.70 times the BMR for immature and adult storks in summer and 1.80 in winter. In this study, all DEE measurements were based on an isotopic method which integrates all costs of activities in captive birds which, obviously, may not be similar to that of free-living birds. Considering this, we used the allometric equation of Williams et al. (1993) on free-living, non-passerine birds to predict a DEE for 3000 g free-living storks of more than double (2600 kJ d^{-1}) the DEE estimated in this study for captive storks in both seasons, denoting the low DEE of these captive birds. Most non-breeding bird species show a higher DEE during winter than in summer (Bryant, 1997; Dawson and O'Connor, 1996; Weathers and Sullivan, 1993), reflecting a significant thermoregulatory cost for cold season survival in temperate-wintering birds (Webster and Weathers, 2000). The mechanisms acting to increase DEE during a colder winter may include increases in basal metabolism (BMR), insulation adjustments, and behavioural adaptations (Cooper and Swanson, 1994; Liknes and Swanson, 1996). In conditions of captivity, the abundance of food requires fewer active searches for meals, which increases the amount of time available for preening and resting. Hence, the energy spent on locomotor activity by storks is minimised, indicating that other components of their energy budgets, such as BMR, in winter might be different. Presumably, the higher DEE of immature male storks may be due to an increase in activity costs associated with agonistic behaviour or voluntary activity (primarily locomotor) in the aviary in order to assure a higher food quantity (Mata, personal observation).

On the one hand, winter improvement of stored energy reserves and insulation seem to be important components of winter acclimatization in European White Storks. On the other hand, seasonal difference in energy expenditure, measured by DLW (25%), suggests that metabolic adjustments may as well significantly contribute to winter acclimatization. In addition to energy reserves and metabolic adjustments, White Storks could have a high resistance to the cold, thanks to effective insulation. White Storks show a low surface-area-to-volume ratio which illustrates the importance of further studies concerning the role of effective insulation of White Storks in effective cold resistance. As a large bird, the White Stork benefits from the thermodynamic advantage of having a slow rate of body heat loss (Calder and King, 1974). However, a White Stork has uninsulated areas where body heat could be lost (head, neck and legs) so the effective role in insulation is not yet known. However, Steen and Steen (1965) determined experimentally that for herons and gulls, less than 10% of the metabolic heat is lost from legs at low ambient temperatures.

4.2. Body mass and composition

Previous studies on captive and free-ranging storks (Hall, et al., 1987; Michard-Picamelot, 1999; Michard, et al., 1995; Michard, et al., 1997) have clearly demonstrated the existence of an annual body mass cycle, characterized by a rise in body mass in mid winter and a decrease at the start of spring corresponding to the onset of the reproduction period. The body mass increment during winter has mainly been attributed to the accretion of body lipids (Michard-Picamelot, et al., 2002). This has also been reported in White Storks by using magnetic resonance imaging spectroscopy (Berthold, et al., 2001), and has been observed in other species that spend winter in Europe (Witter and Cuthill, 1993). This is usually explained by the need for the bird to meet the potential energy deficits arising both from thermogenesis and insufficient food resources (Andrews, 1995). Compared to small birds, large birds (200 g) such as the White Stork (3.5–4 kg) have more capacity to store energy reserves and, hence, greater ability to fast in cold environments (Cherel, et al., 1988; Groscolas, 1990). Furthermore, Mata et al. (2001) found that the high level of lipid stores and the rate of lipid utilization in winter enable White Storks to tolerate induced fasting periods of approximately 28 days during a mild winter. In the current study, the winter adiposity of White Storks was about 31%, a higher value than that of other orders, such as Anseriformes, (12–17%, Ely and Raveling, 1989) and Sphenisciformes, (18–25%, Cherel, et al., 1994; Robin, et al., 1988). This leads us to the conclusion that this high winter adiposity greatly increases the probability for these birds to survive long periods of food deprivation.

4.3. Age and sex

In this study, the winter fat mass increase in adult White Storks was accompanied by a small increase in lean body mass (4%, Fig. 1b). Similarly, a study based on data from the body composition of accidentally dead adult storks (Michard-Picamelot, et al., 2002) concluded that the increase in body mass in winter was mainly due to a change in fat mass, without significant change in protein mass. Contrary to what was currently reported in migrant flapping species, the constancy in protein content throughout the year could be a characteristic of soaring birds like White Storks that do not need to increase their muscle protein prior to migration (Berger and Hart, 1974; Pennycuick, 1975). The LBM increase in adult storks may be related to preparation for the reproductive season.

The fact that female adult and immature White Storks were smaller than males in our study (11%) confirmed the presence of a strong sexual-size dimorphism in this species which has already been observed in a previous study (Michard-Picamelot, et al., 2002). Although presenting the same pattern of body mass seasonal variation, both adults and immatures do not show the same variations in body composition. Contrary to what was observed in adults, LBM increased gradually in both male and female immatures between summer I and summer II, reaching nearly the same level as that of adults in summer II. This suggests that in three year-old storks, muscle mass had not yet reached complete development, even if these young storks had a similar size and body weight as adults (Gangloff and Gangloff, 1987; Michard-Picamelot, 1999). The protein accumulation would ensure the subsequent process of reproduction (Ankney, 1984).

Despite their difference in physiological status, the adiposity during winter was very similar in immatures and adults (32 vs 30%). This result contrasts markedly with the adiposity values measured during summer I, which were much higher in immatures than in adults (29 vs 23%). Therefore, the level of adiposity reached during winter does not appear to depend on age classes. For these birds, it could correspond to an optimum level of lipid stores, which would be sufficient to face winter constraints whilst minimising a gain in weight (a potential handicap for flight).

Due to human intervention, the Alsatian population of White Storks has doubled compared to the beginning of the last century (S. Massemin, personal com.). However, the successful settlement of young storks is still too recent to conclude on the long-term viability of this winter acclimatization. An attentive follow-up of these settled storks is therefore required to specify the relationship between phenotypic plasticity (metabolic flexibility) (Piersma and Lindström, 1997) and environmental variation of their new living conditions. In this context, the double labeled water method used in this study is quite a suitable tool, since it allows a non-invasive determination of both body composition and energy expenditure throughout the year, on the same individuals.

Our results have established that for both adult and immature storks, the increase in total body mass in winter is mainly attributed to an increase in fat mass. However, the most unexpected result of this study concerns the absence of a significant cost of thermoregulation during winter for adult males and both immature and adult females. It could be the mere consequence of mild winter temperatures and the large accumulation of fat in subcutaneous tissues ensuring efficient heat insulation. Even if this accumulation of fat cannot be attributed specifically to the endogenous cycle (including migration), or to acclimatization for the winter period in settled White Storks, we can nevertheless advance that the population of White Storks has the capability to cope with mild winters in Europe. This finding is of a particular interest in the context of the idea that persistently warm climatic conditions favour an increase in the size of a settled White Stork population in Europe (Archaux, 2003). Based on physiological parameters, this study shows that settled White Storks are able to cope with mild winter periods when they are artificially provided with food. It is suggested, within the context of preserving favourable White Stork habitats, that a project should therefore be set up for the breeding area. Additional research remains necessary to determine if the patterns presented here occur in free-living storks.

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