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TECHNICAL NOTE

Flight muscle shape reliably predicts flight muscle mass of migratory songbirds: a new tool for field ornithologists

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Abstract The pectoral muscle is the biggest organ within a passerine bird. It provides flight locomotion and is known to act as a protein source during periods with increased protein demands or decreased protein availability. The mass of the flight muscle is dynamic and changes during juvenile growth, reproduction, seasonal acclimatization, fasting and migration. Thus, a tool that accurately and non-invasively quantifies this phenotypic flexibility in flight muscle mass is of interest to ornithologists. We provide a calibration and validation of a "muscle meter" device designed to accurately measure the shape of the flight muscle. For two species of different size, the European starling (*Sturnus vulgaris*) and the garden warbler (*Sylvia borin*), we compared the accuracy and precision of different linear regression models for predicting flight muscle mass. The multifactorial linear regression model with the most support for both species included "muscle meter score" (mm_{score}), tarsus length and body mass (m_b), although a simpler model with mm_{score} and m_b had as much support for predicting flight muscle mass of European starlings. A validation exercise revealed that flight muscle mass of these two species could be estimated with a relative error of about 3%. The muscle meter is a simple device, easy and quick to handle, that can reliably and non-invasively estimate flight muscle mass of captive and wild birds when used in conjunction with standard measurements of tarsus length and m_b.

Keywords Calibration · Validation · Pectoral muscle · Body condition · Passerine

Zusammenfassung Der große Flugmuskel (*Musculus pectoralis*) ist das größte Organ im Singvogelkörper. Er ermöglicht das Fliegen, stellt aber auch eine körpereigene Proteinquelle dar, die bei erhöhtem Proteinbedarf oder reduzierter Proteinverfügbarkeit genutzt wird. Die Masse des großen Flugmuskels zeigt ein dynamisches Verhalten und ändert sich beispielsweise während des jugendlichen Wachstums, der Reproduktion, der jahreszeitlichen Anpassung, des Fastens und des Zuges. Ein Hilfsmittel, zu einer exakten und nicht-invasiven Erfassung dieser phänotypischen Flexibilität des Flugmuskels ist deshalb von großem Interesse für Ornithologen. Hierzu stellen wir hier das 'Muskel-Meter' sowie seine Kalibrierung und Validierung, zur exakten Messung der Form des Flugmuskels vor. Bei zwei unterschiedlich großen Vogelarten, dem Europäischen Star (*Sturnus vulgaris*) und der Gartengrasmücke (*Sylvia borin*), haben wir multifaktorielle lineare Regressionsmodelle auf ihre Genauigkeit hinsichtlich einer präzisen Voraussage der Flugmuskelmasse überprüft. Das Regressionsmodell mit der besten Anpassung an die empirischen Daten beider Vogelarten beinhaltet den

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'Muskel-Meter Score' (mm_{score}), die Tarsuslänge und die Körpermasse (m_b). Das einfachere Modell mit mm_{score} und m_b erzielt eine ähnliche Übereinstimmung, allerdings nur für den Europäischen Star. Eine Validitätsprüfung zeigt, dass die Flugmuskelmasse der beiden Arten mit einem relativen Fehler von 3% abgeschätzt werden kann. In Kombination mit Standardmessungen, wie der Tarsuslänge und der Körpermasse, ist somit das Muskel-Meter bestens für eine rasche und genaue, nicht-invasive Abschätzung der Flugmuskelmasse von Wildvögeln und Vögeln in Tierhaltung geeignet.

Introduction

The pectoral muscle in birds amounts to 10–25% of body mass for most actively flying species with some extreme cases of even more than 30% (Hartman 1961). It is thus the biggest single organ within a passerine's body. Besides its main function for flight locomotion, it serves as a protein source during times with high protein demands or low protein intake (Jones and Ward 1976; Piersma and Jukema 1990; Houston et al. 1995; Cottam et al. 2002; for review, see: Piersma and Lindström 1997; Bauchinger and Biebach 1998; Jenni and Jenni-Eiermann 1998). The pectoral muscle generates the force for the down stroke of the wing, while the supracoracoideus muscle lifts the wing. Both muscles together are often referred to as flight muscle. Because the pectoral muscle roughly contributes to about 90% of the flight muscle mass, with the remainder being associated to the supracoracoideus muscle (Hartman 1961), the term flight and pectoral muscle are often used interchangeably.

Mass of the flight muscle in birds changes during the annual cycle and especially during growth, migration, egg laying, molt and seasonal acclimatization (for review see: Piersma and Lindström 1997; Bauchinger and Biebach 1998; Cottam et al. 2002; Piersma and Drent 2003; Dietz et al. 2007; Swanson 2010). In general, flight muscle mass is positively related to intraspecific variation in body size (Calder 1984). Flight muscle mass often tracks body mass (m_b) during various conditions like flight, starvation and re-feeding (Lindtsröm et al. 2000), during the annual cycle (Dietz et al. 2007) and during migration (Fry et al. 1972; Marsh 1984; Bauchinger and Biebach 2001; Dietz et al. 2007). However, changes in flight muscle mass are not always associated with changes in body mass. Eared grebes (*Podiceps nigricollis*) increased m_b while flight muscle mass decreased when they were flightless at an autumn staging ground, and then m_b decreased while flight muscle mass increased just prior to the grebes leaving the staging area (Gaunt et al. 1990, Jehl 1997). Migratory garden warblers (*Sylvia borin*) during migration showed a general positive relationship between flight muscle mass and m_b when sampled immediately before and after long flight periods (Bauchinger and Biebach 2001). However, garden warblers arriving at stopover sites at the edge of an ecological barrier and prior to the flight across this barrier had relatively heavy flight muscle mass even though m_b was relatively low (Bauchinger and Biebach 2005).

Several methods are available for non-invasively estimating flight muscle mass of larger birds, although those used for songbirds provide only qualitative indices of flight muscle mass that are subjectively assessed. Measuring flight muscle thickness by use of ultrasound has proven a useful method to estimate flight muscle mass in different wader species (Lindtsröm et al. 2000; Dietz et al. 1999a, b). Dietz et al. (1999a) report that flight muscle mass of two wader species can be estimated with an individual error of 20–25%. Contouring the shape of the muscle with solder wire over a defined point at the *Carina sterni* provided a reliable estimate of flight muscle mass in gulls (Bolton et al. 1991) and geese (Nyeland et al. 2003). Selman and Houston (1996) molded the shape of the flight muscle of passerine birds into a gel used for dental imprints and quantified the volume of the flight muscle from the negative imprint. An index of flight muscle size of geese and songbirds was obtained by scoring the shape of the flight muscle (e.g., concave, straight, convex, bulging) on both sides of the *Carina sterni* (Bairlein 1995; Nyeland et al. 2003). The advantage of such an index is that the muscle score is simple, repeatable and fast, and can thus be performed on hundreds of individuals at ringing stations. However, such indices provide an estimate of only relative flight muscle shape and not flight muscle mass (but see for geese: Nyeland et al. 2003). Other techniques measure flight muscle thickness or shape, but require time and handling effort, more than one person or costly equipment, which limits their usefulness especially for studies of free-living birds (see Bairlein 1995).

Herein, we describe a new technique to estimate flight muscle shape that, when combined with other size measures, can quickly and reliably estimate flight muscle mass of free-living wild-caught birds. The muscle meter device is simple, easy to handle by one person and provides fast yet accurate measurements of the flight muscle shape. For two species of different size, the European Starling (*Sturnus vulgaris*) and the garden warbler (*Sylvia borin*), we compared the accuracy and precision of different linear regression models for predicting flight muscle mass. Our data sets were as heterogeneous as possible, providing maximum variability in terms of structural size, m_b and flight muscle mass in order to demonstrate the predictability of flight muscle mass by application of muscle meter measurements.

Materials

Accurate use of any non-invasive technique for estimating body composition of free-living birds requires performance of a validation study (Scott et al. 2001). One validation approach involves building predictive models for estimating flight muscle mass using a subset of birds (calibration) and then using the models to predict flight muscle mass in another subset of birds that were not used to develop the predictive models (validation). We predicted flight muscle mass (m_{fm}) of European starlings (*Sturnus vulgaris*) and garden warblers (*Sylvia borin*) given their body mass, tarsus length and muscle meter measures of their flight muscle shape. Body mass of European Starlings ranges between 60 and 90 g (Snow et al. 1998), whereas body mass of garden warblers ranges between 15 and 25 g (Bairlein 1987; Bairlein 1991), with extreme values beyond those ranges for both species. All birds used in this contribution were part of investigations on migration and flight physiology, and so spanned the range of documented body mass (and flight muscle mass). We captured 116 starlings during August in Ontario (43°10′N; 81°19′W), Canada, and maintained them for up to 4 months in captive facilities at the AFAR (Advanced Facility for Avian Research) facility at University of Western Ontario. A total of 45 garden warblers were used in this study with 14 birds captured in Turkey (36°40′N; 33°05′E) during the autumn migration (Biebach 1998; Bauchinger and Biebach 2001) and 31 birds captured on the Crimean peninsula (45°25′N; 32°32′E) in the Ukraine during the spring migration. Data sets for both species were decidedly heterogeneous with respect to sex, body size and body mass.

For all birds we measured tarsus length, (Svensson 1992), m_b and the shape of the flight muscle at a defined location (subsequently, mm_{score}). Figure 1 shows the muscle meter device and describes the measurement method in detail. We took three consecutive measures of mm_{score} for each starling and then used the mean of these three measures for subsequent modeling, whereas we took only one measure of mm_{score} for each garden warbler. Subsequently, birds were anesthetized under Isoflurane and killed by cervical dislocation and the left pectoral and supracoracoideus muscles were dissected. Fresh mass of the muscles was determined immediately for starlings and within weeks upon storage in liquid nitrogen for warblers (for methods see Bauchinger and Biebach 2001). For calculation of flight muscle mass (m_{fm}) of each bird we combined the samples of the pectoral and the supracoracoideus muscles, multiplied those by two and hereafter refer to this as m_{fm}. We calculated repeatability for the measurements of mm_{score} by use of the muscle meter according to method described by Lessels and Boag (1987).

Procedure for developing predictive models

The complete data set for each species was used to build regression models with flight muscle mass as the dependent variable and all possible combinations of the following independent variables: tarsus length, mm_{score} or m_b. We used the r^2-value and the Akaike information criterion (AICc) for low sample size to evaluate which model(s) explained the most variance and had the highest level of support (Burnham and Anderson 2002). All data were tested for normality (*K–S* test) prior to regression analysis. Linear regression models were used to describe the relationship between the independent variables to m_{fm}, because cubic and quadratic models did not explain more of the variance and were more parsimonious.

We estimated the accuracy and precision of the predictive models using a validation approach. We randomly selected 88% of the birds of each species (105 European starlings and 39 garden warbler) and built regression models with flight muscle mass as the dependent variable and all possible combinations of the following independent variables: tarsus length, mm_{score} or m_b. We then estimated the m_{fm} of the remaining birds (11 European starlings and 6 garden warblers) using these regression models built with the 88% subset of birds. To ensure that the birds used to build each model spanned the full range of body mass for that species, we assigned individuals with the two most extreme values for tarsus length, mm_{score} and m_b of each of the two species to the calibration group used to build the model, and randomly selected the remaining individuals for the calibration and the validation group. We estimate the absolute and relative error terms for the predictive models for the comparison between the estimated m_{fm} and the m_{fm} value determined after dissection by weighing the tissue, because coefficients of determination are not always indicative of error when evaluating the usefulness of regression equations (e.g., Skagen et al. 1993). Absolute error was calculated as |predicted-actual|, and relative error as [100 × (|predicted—actual|)/actual]. We used PASW Statistics (version 18) for the analysis.

Results

Distribution and range of measurements

We used untransformed tarsus length, mm_{score} and m_b to predict m_{fm} because these data were normally distributed (*K–S* test $P > 0.40$ for all parameters and both species). For starlings ($n = 115$), the range for tarsus length was 5.1 mm (min. to max. 26.7 to 31.8 mm), the range for mm_{score} was 3.46 mm (−6.27 to −2.79 mm), the range for m_b was 26.2 g (55.3–81.5 g), and the range for flight

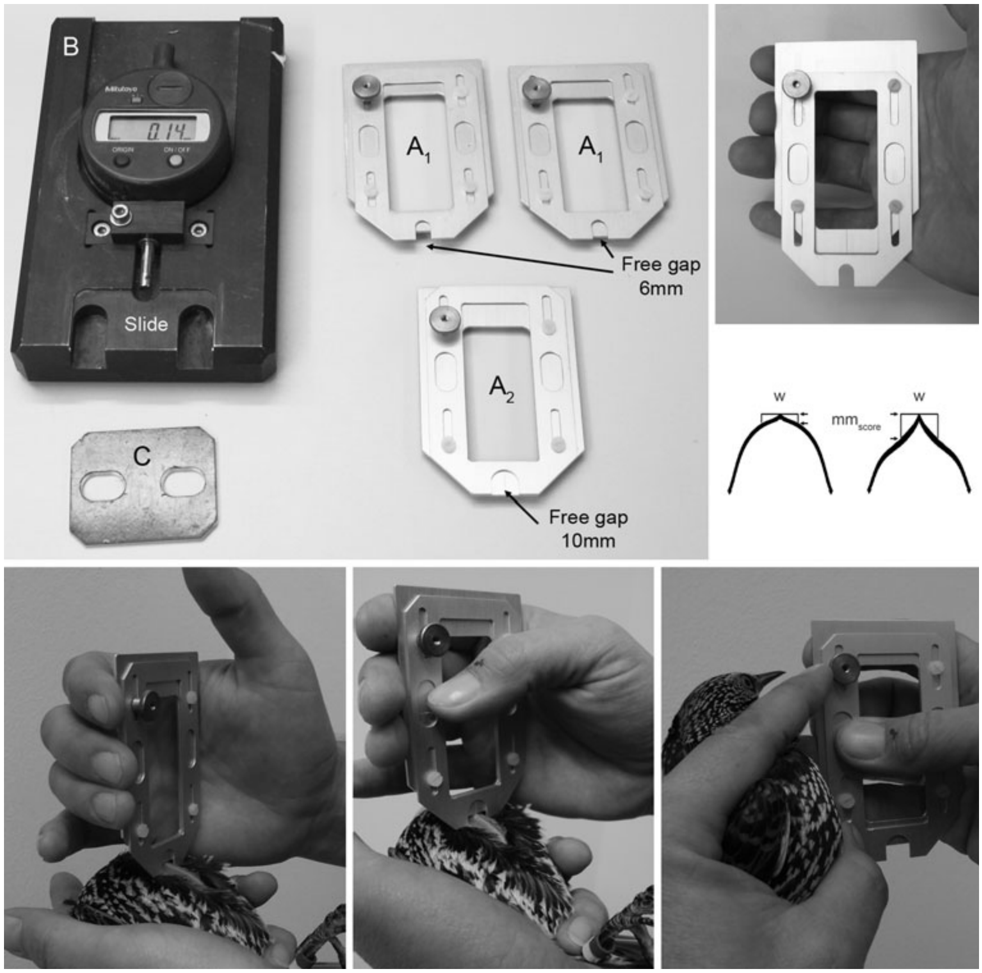

Fig. 1 Muscle meter and mounted caliper used to measure the shape of the flight muscle. *Left top* image shows three muscle meter devices of which two have a free gap of 6 mm (A_1) and one has a free gap of 10 mm (A_2). The larger gap is used for bigger passerines birds like starlings, while the 6-mm gap is used for measurements on smaller sized birds, like the garden warbler. Picture on the top right demonstrates handling of the muscle meter, with the front panel sliding freely above the back panel. The *front panel* is then placed perpendicular on the flight muscle of a bird, which is held horizontally in the alternate hand (see *bottom left picture*). The location where the muscle meter is placed on the flight muscle is defined as the middle of the *Carina sterni* between its posterior and anterior end points. The muscle meter is lowered perpendicularly onto the flight muscle until the *back panel* rests on the *Carina sterni*. This position of the muscle meter is now secured by pressing the free thumb of the hand that is holding the muscle meter against the front panel thereby locking the position of the muscle meter (see *bottom middle picture*), and subsequently by closing the screw with a finger from the hand that holds the bird (see *bottom right picture*). The distance measured between the back panel (= level of the *Carina sterni*) and the front panel that measures the shape of the muscle (mm_{score} in the muscle sketch) is now fixed at the lower part of the muscle meter. This distance is similar to the distance between the two panels at the upper end of the muscle meter, because both panels have equal length. The upper part of the muscle meter is now pushed gently into the slide at the measuring device (*B*) that holds a mounted caliper, and the final mm_{score} is read from the caliper display. The caliper is mounted onto a metal block to provide stability and allows the measurement to be performed with one hand only, with the other hand still holding the bird. Before a set of measurements is recorded the calibration tool (*C*) is pushed into the slide, and the value is set to zero to calibrate the caliper and its position in the device. The caliper is mounted in the measuring device (*B*) in a way that the zero point already represents an input tension, i.e., without the calibration tool (*C*) the caliper reads a value > zero. We used an input tension of 0.14 mm; actual values for the mm_{score} are read as negative values so that increasing values signify increasing muscle bulging. Using three repeated mm_{score} measures to create a mean value reduces the likelihood of odd values to find their way into the data set

muscle mass was 7.44 g (10.86–18.30 g). For garden warblers ($n = 45$), the range for tarsus length was 2.7 mm (min.–max., 18.9–21.6 mm), the range for mm_{score} was 1.75 mm (−3.74 to −1.99 mm), the range for m_b was 12.8 g (14.7–27.5 g), and range for flight muscle mass was 1.26 g (2.23–3.49 g).

In European starlings measurements of mm_{score} showed a repeatability (r) of 0.79 ($r = s_A^2/(s^2 + s_A^2)$, where $s_A^2 = 0.39$

is the among group variance component and $s^2 = 0.10$ the within-group variance component.

Calibration and Akaike information criterion

We compared r^2-values and Akaike information criteria for the seven linear regression models for each species with flight muscle mass as the dependent variable and all possible combinations of the following independent variables: tarsus length, mm_{score} and m_b (Table 1). All starlings ($n = 116$) and garden warblers ($n = 45$) were used to build these regression models. r^2-values increased and Akaike information criteria decreased from a single factor to multiple factor regression analysis, and in both species the linear regression model combining the three factors of tarsus length, m_b and mm_{score} had the highest r^2-values and substantially more support based on Akaike information criteria. For European starling, both a two-factor linear regression model and the three-factor linear regression model provide good support based on Akaike information criteria (please see below). The three-factor linear regression model for predicting flight muscle mass (m_{fm}) was as follows for each species:

European starling:

$$m_{fm} = 3.797 + (0.886 \times mm_{score}) + (0.189 \times m_b) + (0.059 \times \text{tarsus length}) \quad (1)$$

Garden warbler:

$$m_{fm} = -1.212 + (0.293 \times mm_{score}) + (0.045 \times m_b) + (0.199 \times \text{tarsus length}) \quad (2)$$

For garden warblers, the three-factor model (Eq. 2) was the only model that was substantially supported, with Δ AICc values of nine and higher for the remaining models (Table 1). For European starlings, both the three-factor model (Eq. 1) as well as the two-factor model (Eq. 3) were substantially supported (Table 1), with Δ AICc values of zero for the two factorial linear regression model (Eq. 3), and a Δ AICc value of 2.9 for the three factorial model (Eq. 2). All other models for the estimation of m_{fm} for European starlings failed to substantially explain variation in these data (Table 1). This most parsimonious two-factor linear regression model for predicting flight muscle mass (m_{fm}) was as follows:

European Starling:

$$m_{fm} = 5.003 + (0.860 \times mm_{score}) + (0.195 \times m_b) \quad (3)$$

Validation

Consistent with the above results from the calibration, when we compared the predicted and actual m_{fm} using the validation subset of both bird species; the absolute and relative errors for estimated m_{fm} were lowest for the three-factor linear regression model (Table 2). Error terms for both species increased with increasing Δ AICc values. For the European starling, mean absolute error increased from 0.39 g for the three-factor model to 1.16 g for the single-factor model that included only tarsus length. For the garden warbler, mean absolute error increased from 0.12 g for the three-factor model to 0.27 g for the single-factor model that included mm_{score}. Mean relative error for the three-factor model was 2.6% for the European starling and 3.1 for the garden warbler, and it increased to 7.8 and 9.1%, respectively, for the single-factor models.

Table 1 Akaike information criteria for different regression models used to predict flight muscle mass for *Sturnus vulgaris* and *Sylvia borin*

Model	r^2	RSS	K	n	AICc	ΔAICc
Sturnus vulgaris						
$Y = mm_{score} + \text{tarsus} + m_b$	0.82	50.0	5	115	−85.3	2.9
$Y = mm_{score} + m_b$	**0.82**	**50.5**	**4**	**116**	**−88.2**	**0.0**
$Y = mm_{score} + \text{tarsus}$	0.62	105.3	4	115	−1.7	86.4
$Y = \text{tarsus} + m_b$	0.74	72.1	4	115	−45.3	42.9
$Y = m_b$	0.73	74.5	3	116	−45.1	43.1
$Y = mm_{score}$	0.54	128.8	3	116	18.3	106.5
$Y = \text{tarsus}$	0.06	258.1	3	115	99.2	187.4
Sylvia borin						
$Y = mm_{score} + \text{tarsus} + m_b$	**0.64**	**1.3**	**5**	**44**	**−143.6**	**0.0**
$Y = mm_{score} + m_b$	0.49	1.9	4	45	−134.3	9.3
$Y = mm_{score} + \text{tarsus}$	0.51	1.8	4	44	−132.7	10.9
$Y = \text{tarsus} + m_b$	0.52	1.8	4	44	−133.6	10.0
$Y = m_b$	0.42	2.1	3	45	−130.5	13.2
$Y = mm_{score}$	0.30	2.6	3	45	−122.5	21.2
$Y = \text{tarsus}$	0.15	3.1	3	44	−111.0	32.7

RSS Residual sum of squares, *K* number of parameters in equation plus slope and intercept, *n* sample size, *AICc* Akaike information criteria for small sample size, *ΔAICc* difference between AICc minimum for each species and the AICc for the respective linear regression model. Regression models with the highest level of support based on ΔAICc are presented in bold

Discussion

Our calibration and validation experiments for two passerines show that using the newly developed muscle meter in conjunction with measurements for whole animal mass and structural size results in reliable estimates of m_{fm} in live birds. In order to obtain estimates for m_{fm} with a relative error margin of about 3%, two additional measurements were included in the linear regression model, namely the tarsus length as a measure of structural size and m_b. Both

Table 2 Accuracy and precision of predictive linear regression models for the estimation of flight muscle (m_{fm}) for European starling (*Sturnus vulgaris*) and Garden warbler (*Sylvia borin*)

Model	r^2	n_{cal}	n_{val}	$m_{fm\ dissected}$		Absolute error (g)				Relative error (%)			
				Mean	SD	Mean	SD	Min	Max	Mean	SD	Min	Max
Sturnus vulgaris													
Y = mm_{score} + tarsus + m_b	0.81	103	11	15.21	1.66	0.39	0.33	0.03	0.99	2.6	2.3	0.2	6.9
Y = mm_{score} + m_b	**0.81**	**104**	**11**	**15.21**	**1.66**	**0.39**	**0.34**	**0.01**	**1.01**	**2.6**	**2.3**	**0.1**	**7.0**
Y = mm_{score} + tarsus	0.59	103	11	15.21	1.66	0.51	0.37	0.02	1.10	3.3	2.2	0.1	6.5
Y = tarsus + m_b	0.73	103	11	15.21	1.66	0.67	0.38	0.10	1.07	4.6	2.7	0.6	7.4
Y = m_b	0.73	104	11	15.21	1.66	0.63	0.34	0.17	0.71	4.3	2.4	1.2	6.1
Y = mm_{score}	0.51	104	11	15.21	1.66	0.65	0.44	0.02	1.25	4.4	3.0	0.1	9.1
Y = tarsus	0.05	103	11	15.21	1.66	1.16	0.99	0.05	3.01	7.8	7.4	0.3	26.1
Sylvia borin													
Y = mm_{score} + tarsus + m_b	**0.61**	**38**	**6**	**3.01**	**0.31**	**0.12**	**0.09**	**0.02**	**0.27**	**4.1**	**3.1**	**0.6**	**8.9**
Y = mm_{score} + m_b	0.47	39	6	3.01	0.31	0.15	0.15	0.00	0.34	5.2	2.4	0.1	13.7
Y = mm_{score} + tarsus	0.51	38	6	3.01	0.31	0.19	0.13	0.03	0.36	6.4	3.9	1.1	10.7
Y = tarsus + m_b	0.47	38	6	3.01	0.31	0.14	0.07	0.03	0.22	4.5	2.4	1.2	7.5
Y = m_b	0.35	39	6	3.01	0.31	0.15	0.06	0.06	0.23	5.2	2.5	2.0	9.1
Y = mm_{score}	0.35	39	6	3.01	0.31	0.27	0.17	0.05	0.49	9.1	6.5	1.7	19.8
Y = tarsus	0.12	38	6	3.01	0.31	0.22	0.19	0.04	0.58	6.9	5.4	1.3	17.0

Estimated flight muscle mass ($m_{fm\ estimated}$) is based on measurements of muscle shape (mm_{score}), tarsus length and body mass (m_b), and compared to the flight muscle determined by weighing following dissection ($m_{fm\ dissected}$). The r^2 values are from models built with a subset of individuals (n_{cal}; ~88% of the total sample size n). Absolute and relative errors give differences between $m_{fm\ dissected}$ (mean, SD, minima and maxima) and the $m_{fm\ estimated}$ for the subset of individuals (n_{val}; ~12% of the total sample size n) used to validate the model. In bold are the models for each species that are most parsimonious, are best supported (see Table 1), and have the lowest absolute and relative error

these additional measures are routinely collected at ringing stations. For European starling use of the mm_{score} in conjunction with m_b already provides reliable estimates of m_{fm}, even without additional measurements of the tarsus length. Whether or not tarsus length is taken into account for estimating m_{fm} does not alter the relative error margins (Table 2). Besides providing a reliable estimate of flight muscle mass of an individual captured once (as commonly occurs at a ringing station), this technique allows repeated measurements of the same individual either in captive studies or in mark re-capture studies in the field.

We used the muscle meter with two different mouth gaps, 10 mm for bigger birds like the European starlings and 6 mm for smaller birds like the garden warbler, and this provided accurate estimates of the m_{fm} with similar relative error (3 and 4%, respectively) when used in conjunction with measurements for tarsus length and m_b. We estimated the m_{fm} of European starlings with 0.39 ± 0.33 g absolute error, which was relatively small given the mean flight muscle of starlings was 14.97 ± SD 5.16 g. Likewise, we estimated the m_{fm} of garden warblers with 0.12 ± 0.09 g absolute error, which was relatively small given the mean flight muscle of these warblers was 2.89 g ± SD 0.29. Although we measured mm_{score} only once for each garden warbler and repeatability for the measurements of mm_{score} is high (0.79 for the starlings), we recommend three repeated measures of the mm_{score}, as done for each European Starling, in order to ensure the accuracy and precision of these estimates.

The accuracy and precision of the muscle meter are sufficient for studying phenotypic flexibility of flight muscle in wild birds. Flight muscle mass of garden warblers decreased on average by 0.51 g during their spring flight across the Sahara desert (Bauchinger et al. 2005), which is more than four times the error that we report for the estimates of m_{fm} from the muscle meter measurements in conjunction with tarsus length and m_b. Similarly, m_{fm} of garden warblers increased on average by 0.27 g during molt while in the African wintering area (Bauchinger and Biebach 2006), which is more than double the error that we report for the estimates of m_{fm} from the muscle meter measurements. Other studies on phenotypic mass changes in garden warblers indicate similar mass changes that could be assessed using the muscle meter (Hume and Biebach 1996; Schwilch et al. 2002). In European starlings the maximum difference in fresh m_{fm} between exercised and unexercised birds amounted to 1.8 g (based on mass for pectoral muscle, Swaddle and Biewener 2000), and this is more than four times higher than the error that we report for the estimates of m_{fm} from the muscle meter measurements. These documented differences for m_{fm} in wild birds or in captive birds subjected to ecologically relevant treatment indicate that phenotypic changes in the flight muscle mass frequently occurs in response to exercise, seasonal acclimatization, migration and/or molt, and that those phenotypic mass changes could be detected by estimation of m_{fm} using the muscle meter.

The muscle meter device has been used to measure the change in flight muscle shape in several studies of birds, although none of these studies predicted m_{fm} from measured mm_{score} because no validation study like that presented here had been completed. For example, Schmidt-Wellenburg et al. (2007, 2008) reported changes in mm_{score} of up to 0.55 mm for rose-colored starlings (*Sturnus roseus*) before and after a 6-h flight in a windtunnel (Schmidt-Wellenburg et al. 2008). Bize et al. (2007) used the muscle meter (mouth gap of 10 mm) to determine changes in flight muscle shape in alpine swifts (*Apus melba*) in response to a prolonged period of inclement weather. They documented a rapid decrease in mm_{score} of about 1.4 mm during inclement weather. European starlings that experienced rich, intermediate or poor foraging environments showed marked differences in mm_{score} of as much as 1.08 mm (Wiersma et al. 2005). Unfortunately, Wiersma et al. (2005) used a mouth gap of 6 mm for their muscle meter, whereas we used a wider mouth gap of 10 mm. Therefore, we are not able to estimate the actual change in muscle mass of the starlings from the data provided by Wiersma et al. (2005). We recommend that standard mouth gaps be used for each species so that results from different studies are most comparable. Clearly, the muscle meter detects significant changes in flight muscle shape that, given validation studies such as ours, can be used to predict changes in flight muscle mass. In concert with a calibration like the ones presented here for European starlings and garden warblers, precise estimates of m_{fm} and m_{fm} changes are possible, which will help to contribute to the understanding of the phenotypic mass changes of the flight muscle.

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