

# Breast muscle variation before and after crossing large ecological barriers in a small migratory passerine (*Sylvia borin*, Boddaert 1783)

Christos BARBOUTIS<sup>1,2\*</sup>, Moisis MYLONAS<sup>1</sup> and Thord FRANSSON<sup>3</sup>

<sup>1</sup> Department of Biology & Natural History Museum of Crete, University of Crete, PO Box 2208, 71409 Heraklion, Crete, Greece

<sup>2</sup> Antikythira Bird Observatory, Hellenic Ornithological Society, 24, Vas. Irakleiou Str., 10682 Athens, Greece

<sup>3</sup> Bird Ringing Centre, Swedish Museum of Natural History, Box 50 007, SE-104 05 Stockholm, Sweden

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During long-distance flights, birds catabolize not only fat but also protein, which results in structural or functional loss as protein is stored in organs. In this study we investigated breast muscle size in relation to body mass in garden warblers *Sylvia borin* before and after crossing the Sahara desert and the Mediterranean Sea in autumn and spring migration, respectively. Breast muscle size was measured by moulding the shape of the muscles of live birds. Samples were collected on the Greek island of Antikythira during spring after the barrier crossing and on Crete during autumn, where garden warblers prepare for barrier crossing on their southward migration. Breast muscle size on Antikythira was significantly smaller than the equivalent size measured on Crete. Breast muscle size followed the changes in total body mass when garden warblers had a low body mass while this relationship disappeared when body mass is above 17.5 g. The combination of low body masses and small breast muscle size of birds that have just crossed the Sahara desert and the Mediterranean Sea and landed on Antikythira indicates a severe protein break down. During extended flights, when fat reserves are insufficient, the use of protein can also be a lifeline to reach the destination. The weak association between body mass and breast muscle size during autumn implies that the increase of breast muscle, as a preparation for the barrier crossing, does not take place en route from breeding sites to the Mediterranean area.

**Key words:** avian migration, garden warblers *Sylvia borin*, stopover, flight muscles, barrier crossing.

## INTRODUCTION

Within the Palearctic-African bird migration system, birds face the challenge of passing a large ecological barrier, consisting of the Sahara desert or both the desert and the Mediterranean Sea. The majority of passerine migrants crossing the above mentioned barrier need to store extensive fuel loads in advance and it is well known that close to the barrier migrants often carry very large fuel loads (*cf.* Fry *et al.*, 1970; Finlayson, 1981; Fransson *et al.*, 2006, 2008). In early studies of bird migration physiology it was believed that fat was the only fuel used (Odum *et al.*, 1964),

while nowadays it is clear that some of the total energy released must come from oxidising protein, to satisfy requirements in the oxidative pathway (Bauchinger & Biebach, 1998; Jenni & Jenni-Eiermann, 1998).

Before the onset of migration several species have been shown to increase size of their pectoral muscles (Fry *et al.*, 1972; McLandress & Raveling, 1981; Marsh, 1984; Driedzic *et al.*, 1993; Lundgren *et al.*, 1995; Dietz *et al.*, 1999), which is the largest organ in birds (Hartman, 1961). Breast muscles, along with other organs, undergo a large reduction during crossing the Mediterranean Sea and the Sahara desert (Bauchinger & Biebach 2001; Bauchinger *et al.*, 2005). Eventually, part of the catabolised proteins after long migratory flights have been shown to be restored during

\* Corresponding author: tel.: +30 2810 393279, e-mail: [barboutischr@gmail.com](mailto:barboutischr@gmail.com)

stopover periods in waders (Gaunt *et al.*, 1990; Piersma, 1998; Piersma *et al.*, 1999a). Restoration in breast muscles during stopover has been shown experimentally in passerines as well (Bauchinger *et al.*, 2005).

As there is no way of storing protein (apart from a small amino acid pool) except in organs themselves, the use of proteins as fuel has potential structural or functional consequences. Variation in protein mass of organs in migrants is considered to be an expression of phenotypic flexibility (Piersma, 1998). This flexibility is considered to be an adaptation allowing animals to successfully undergo a wide range of conditions and life-history events (Piersma & Lindström, 1997). Several mutually nonexclusive hypotheses have been suggested to explain the organ changes during migration (for review see Evans, 1992; Lindström & Piersma, 1993; Biebach, 1996; Klaassen, 1996; Bauchinger & Biebach, 1998; Jenni & Jenni-Eiermann, 1998; Klaassen *et al.*, 2000). According to these hypotheses, organ tissue may act as a multifunctional protein resource during negative nitrogen intake or may change, mainly for the flight muscles, in close relationship to the changing body mass. Recently Bauchinger & McWilliams (2009) expressed an alternative non-functional explanation for the vast variation in organ size during migration, proposing that the variation is mainly explained by the tissue-specific turnover rate.

We studied the relationship between the breast muscle size and the total body mass in garden warblers using data from a final stopover site on Crete before crossing the Mediterranean Sea and the Sahara desert during autumn and data from a stopover site, Antikythira, just after crossing the above mentioned barrier during spring. All measurements were obtained using a non-invasive technique.

## MATERIALS AND METHODS

This study was carried out on the islands of Antikythira, Greece (35°51'N, 23°18'E), during spring and on central Crete (35°03'N, 25°13'E), during autumn (Fig. 1). During spring, garden warblers captured within the regular ringing activities organized by the Hellenic Ornithological Society and the Hellenic Bird Ringing Centre at Antikythira Bird Observatory were studied. Trapping on Antikythira took place between 4th April and 25th May 2008 and between 25th March and 25th May 2009. Birds trapped on Antikythira represent individuals that have just passed the ecological barrier of the Sahara desert and the Mediterranean Sea. In contrast garden warblers trapped on central Crete represent birds preparing for the southward passage over the ecological barrier (Fransson *et al.*, 2008). On Crete garden warblers were attracted

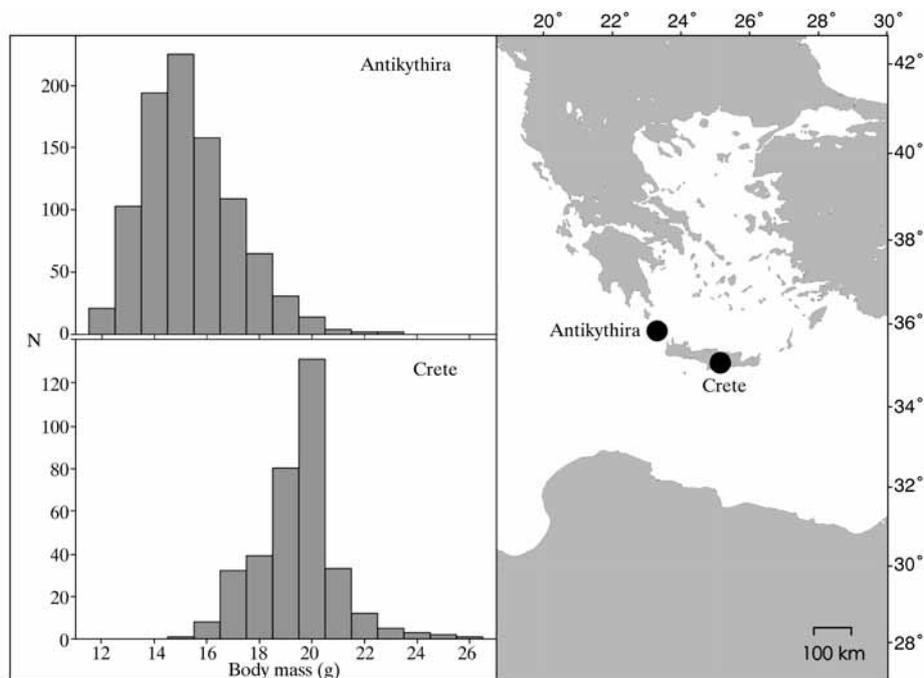


FIG. 1. Location of the study sites and distribution of body mass of birds trapped on Crete during autumn and on Antikythira during spring.

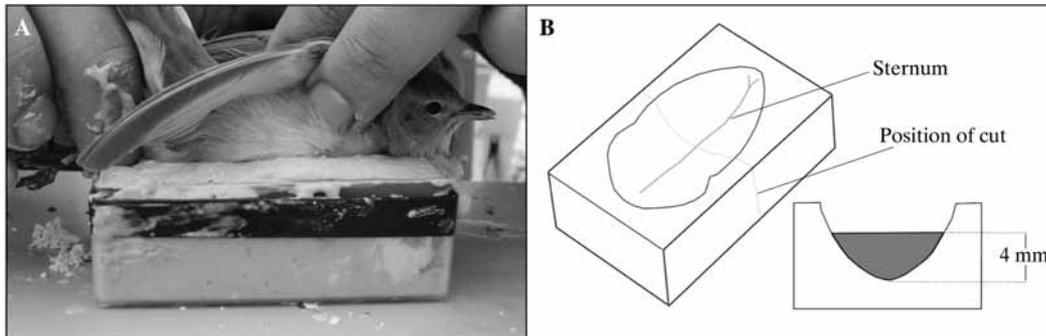


FIG. 2. Moulding process (A) and illustration of the mould (B) as well as cutting point and area estimated and used as breast muscle index.

by tape lures and trapped in mist-nets from dawn until noon between 21st August to 16th September 2008 and 24th August to 8th September 2009. By using tape lures we achieve an overrepresentation of individuals that have just arrived to the study site (Schaub *et al.*, 1999; Fransson *et al.*, 2008). Trapped birds at both sites were ringed and weighed using a digital balance to the nearest 0.1 g. Wing length (maximum chord), was recorded to the nearest 1 mm according to Svensson (1992) as a measurement of size.

An index of the size of the pectoral muscle of the bird was measured using a body mould in dental alginate (Selman & Houston, 1996; Fig. 2A). Every mould obtained was cut dorsoventrally in the middle of the pectoral muscle. Four imprints were taken from each mould (two from each half). A horizontal line was drawn at the perpendicular distance of 4 mm from the position of the keel of each imprint (where 4 mm being used as a standard measure of the average keel depth). The enclosed area representing the cross-sectional area of the flight muscles was then digitalized to be measured. The mean value for the four cross-sectional areas (measured to the closest 0.01 mm<sup>2</sup>) from each mould calculated for every individual and will hereafter be referred as breast muscle index (Fig. 2B). Before making a mould, we examined each bird, thereby confirming that subcutaneous fat did not cover the pectoral which would have given an incorrect size estimate. The cross-section pectoral muscle area established with this moulding technique has been shown to correlate with pectoral muscle lean dry mass in zebra finches *Taeniopygia guttata* (Selman & Houston, 1996).

Twenty-two birds were moulded between 19th April and the 5th of May 2008 and nine birds between 19th April and the 14th May 2009. In total 30 birds were moulded on Crete, eighteen between 1st and 16th September 2008 and twelve between 1st and 8th

September 2009.

To examine relationships between the breast muscle index and body mass of the moulded birds, we developed a linear regression model and compared it with cubic, quadratic, and broken-stick regression models. The best-fitting model was detected using the Akaike information criterion (AIC) corrected for small sample size (AICc) while minimizing the number of parameters in the final model (Burnham & Anderson, 1998). The models were ranked by AICc and the model with the lowest value was chosen as the final model. It was accepted as more parsimonious than other lower-ranking models if the difference in AICc was greater than 2 (Burnham & Anderson, 1998). When normality was met, parametric tests were used, otherwise non-parametric tests were performed.

## RESULTS

In total, 942 garden warblers were trapped on Antikythira during spring of 2008 and 2009. The mean body mass of garden warblers caught on Antikythira was  $15.83 \pm 1.79$  g ( $n = 926$ ). On Crete, 348 individuals were trapped during autumn of 2008 and 2009, with an average body mass of  $19.68 \pm 1.52$  g ( $n = 348$ ).

The mean body mass of birds moulded on Antikythira (spring) was  $15.58 \pm 1.53$  g and varied between 13.3 and 19.2 g (Fig. 3). The equivalent mean value for birds on Crete (autumn) was  $19.97 \pm 1.94$  g and varied between 16.7 and 26.3 g. The mean body mass of birds captured on Antikythira and birds moulded on Antikythira did not show any significant difference (Mann-Whitney U;  $z = -0.563$ ,  $p = 0.57$ ). Likewise the mean body mass of birds captured on Crete and birds moulded on Crete did not show any significant difference (Mann-Whitney U,  $z = -1.014$ ,  $p = 0.31$ ). The mean body mass was significantly different between

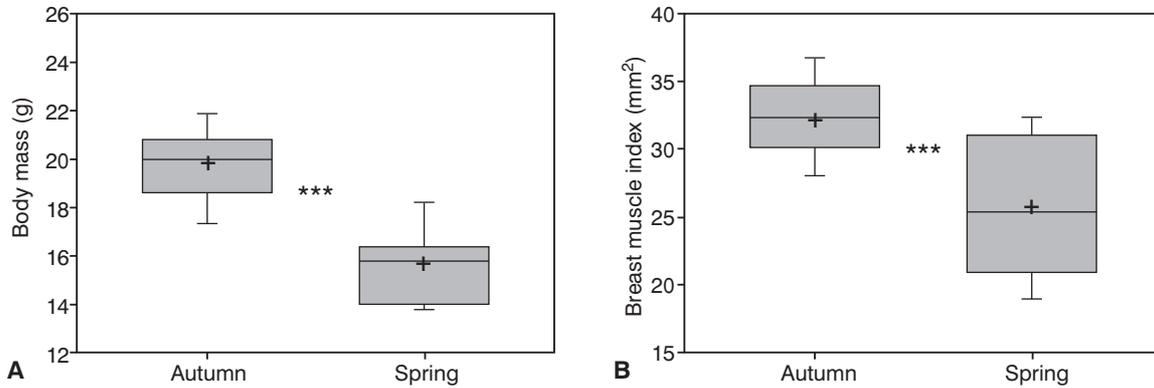


FIG. 3. Box plot of A) body mass and B) breast muscle index of birds moulded, during spring and autumn, respectively and their seasonal comparison. Mean values are shown by +, the lower edge of the box is the 25th percentile, the upper edge is the 75th percentile and median values being the inner line of the graph, \*\*\*:  $p < 0.001$ .

birds moulded on Antikythira and Crete ( $t_{59} = 9.82$ ,  $p < 0.0001$ ). The breast muscle index was significantly different ( $t_{46.4} = 6.4$ ,  $p < 0.001$ ) between birds that had just crossed the barrier (Antikythira during spring:

$25.61 \pm 5.26 \text{ mm}^2$ ,  $n = 31$ ) and birds preparing to cross it (Crete during autumn:  $32.57 \pm 2.83 \text{ mm}^2$ ,  $n = 30$ ; Fig. 3). The breast muscle index varied between 16.09 and 34.88  $\text{mm}^2$  on Antikythira (spring) and between 27.06 and 37.39  $\text{mm}^2$  on Crete (autumn). The relationship between the breast muscle index and body mass was best fitted with a broken-stick relationship model (Table 1). A broken-stick relationship or continuous piecewise regression can be described by the following equation:

TABLE 1. The model selection table of the relationships between the breast muscle index and body mass

Model	k	$\Delta\text{AICc}$	$R^2$
Linear	2	35.23	0.60
Broken Stick	3	0.00	0.79
Quadratic	3	13.58	0.73
Cubic	4	7.26	0.76

k = number of estimable parameters

$$\begin{aligned} bi_1 &= \alpha + \beta_1 * bm, \\ bi_2 &= bi(X_0) + \beta_2 * (br - X_0), \\ bi &= IF(bm \leq X_0, bi_1, bi_2) \end{aligned}$$

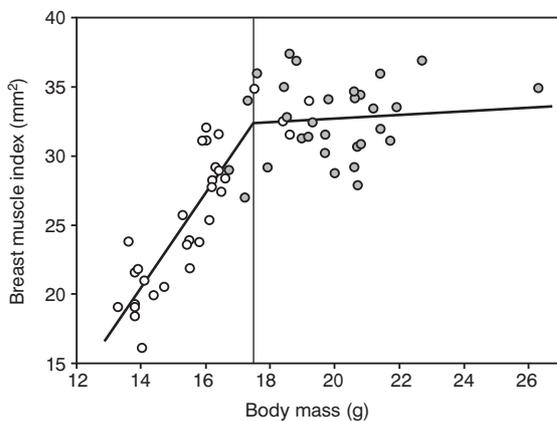


FIG. 4. The relationship between the breast muscle index and body mass of birds moulded. A two-phase regression line is shown ( $bi_1 = -27.83 + 3.449 * bm$ ,  $bi_2 = 32.5275 + 0.2749 * (br - 17.50)$ ,  $bi = IF(bm \leq 17.50, bi_1, bi_2)$ ;  $F_{2,57} = 26.33$ ;  $p < 0.0001$ ;  $R_2 = 0.790$ ) with the estimated breakpoint of  $17.50 \pm 0.39 \text{ g}$ . Birds moulded during spring on Antikythira are presented with open circles and birds moulded during autumn on Crete with filled circles.

where (br) is the breast muscle index and (bm) the body mass,  $\alpha$  is the intercept (in our case  $\alpha = -27.83$ ). Additionally  $\beta_1$  is the slope of the first (left) part ( $\beta_1 = 3.449$ ),  $\beta_2$  is the slope of the second (right) part of the regression ( $\beta_2 = 0.2749$ ) and  $X_0$  is the estimated breakpoint between the two phases ( $X_0 = 17.50 \pm 0.39 \text{ g}$ ). The overall fit of the broken-stick model was good ( $F_{2,57} = 26.33$ ,  $p < 0.0001$ ,  $R^2 = 0.79$ ). Above the breakpoint, the slope of the regression line was not significantly different from zero ( $t = 0.46$ ,  $p = 0.8$ ) indicating that the breast muscle size is not affected by changes in total body mass, at least not in the range of body mass studied (Fig. 4).

## DISCUSSION

As it is deduced from the large variation of breast muscle index (Fig. 3), the actual breast muscle itself is variable in size. On average, before crossing the Mediterranean Sea and Sahara desert the breast muscle index is 21.4% larger compared with birds that

have just passed the above mentioned barrier during spring. Furthermore the variation in breast volume during spring is large, which is not the case for autumn (Fig. 3B). As it is revealed from the broken stick relationship, when garden warblers have a relatively low body mass, the breast muscle size follows the change in body mass while this pattern seems not to be followed when birds reaches the body mass of 17.5 g, since the slope of the regression line above this point is not significantly different from zero. Thus, the existing hypothesis that flight muscle changes should follow body mass changes (Evans, 1992; Lindström & Piersma, 1993; Lindström *et al.*, 2000) is not followed in small passerines, such as the garden warbler (Bauchinger & Biebach, 2005), at least not in the whole range of its body mass. The breast muscle shows a significant reduction after the migratory flight across the Sahara desert, as revealed from the relationship between breast muscle index and body mass in birds arriving to Antikythira (Fig. 4). Similar changes in the flight muscle have been recorded for garden warblers in earlier studies (Biebach, 1998; Bauchinger & Biebach, 2001; Bauchinger *et al.*, 2005) and other species as well (Gaunt *et al.*, 1990; Åkesson *et al.*, 1992; Jehl, 1997; Bauchinger & Biebach, 1998; Piersma, 1998; Piersma *et al.*, 1999a, b; Schilch *et al.*, 2002). Breast muscles accounts for about 10-25% of body mass (Hartman, 1961) and therefore could serve as a protein resource during long flights (Klaassen, 1996). Throughout long migratory flights, when birds carry appreciable fat stores, protein usage as fuel is low and lipid utilisation is high as happening during phase II of fasting (Cherel *et al.*, 1988; Schilch *et al.*, 2002).

When current fat stores drop below a critical level during fasting a progressively and greatly increased protein utilisation is taking place called phase III of fasting (Cherel *et al.*, 1988). A similar pattern, concerning fuel utilisation, occurs during endure migratory flights (Jenni *et al.*, 2000; Schilch *et al.*, 2002). In garden warblers, this critical body mass, after which the increased protein utilisation is taking place, is close to 17.5 g as found out from the data presented in this study. During this face proteins will derive from all organs, but especially from breast muscles (Schilch *et al.*, 2002) and there is a steep drop in the breast muscle index and thus in the breast muscle size, which most probably will result in reduced flight capacity. When the body mass of a garden warbler approaches 17.5 g an average bird is roughly left with a fuel load of 16.7% of body mass without fuel (considering the average body mass without fuel to be 15

g; Bairlein, 1987; Grattarola *et al.*, 1999).

Depletion of fat reserves and structural muscles as flight muscles, are expected in birds that have just crossed large ecological barriers (Biebach, 1998; Bauchinger & Biebach, 2001; Bauchinger *et al.*, 2005). Arriving depleted after the barrier crossing in this region seems to be a general pattern in garden warblers and the mean body masses of birds trapped are among the lowest found in literature for birds in the same migratory step (Bairlein, 1991; Grattarola *et al.*, 1999; Messineo *et al.*, 2001) indicating that the extent of protein brake down during migratory flight is very high for birds landing on Antikythira. Apart from the flight muscles, nutritional organs are clearly reduced (Biebach, 1998; Schilch *et al.*, 2002) and this can lead to decreased digestive capacity (Lee *et al.*, 2002) and lower feeding and digestion rates (Hume & Biebach, 1996; Karasov & Pinshow, 2000) even though it has been shown that birds can maintain high digestive efficiency (Bauchinger *et al.*, 2009; Tracy *et al.*, 2010). The large extent of breast muscle needed to be restored before continuing the migration towards the breeding grounds and the possible low refuelling rates might reduce the species overall migration speed with direct consequences to fitness, as there is strong selection pressure on migrants to minimize time used for migration (Hedenström & Ålerstam, 1997).

Contrary to birds that have just crossed the desert and Mediterranean Sea, in birds sampled during autumn the breast muscle index did not reveal any clear relationship with the body mass. The same pattern was found in garden warblers close to the edge of the Mediterranean Sea and the Sahara desert during autumn migration at another site (South Turkey; Bauchinger & Biebach, 2005). Both Crete (this study) and South Turkey are the last stopover sites before the barrier shaped by the Mediterranean Sea and the Sahara desert. Garden warblers have been shown to increase their average body mass along the migration route south in autumn and this increase was found to be larger in eastern birds (Bairlein, 1991; Schaub & Jenni, 2000). The successive increase in body mass towards the south has been seen as a preparation for the oncoming barrier crossing and the larger increase in eastern birds as a result of having to cross the most inhospitable area of the Sahara (Schaub & Jenni, 2000). Most of the fuel needed for the barrier crossing is, however, accumulated at their last stopover (Fransson *et al.*, 2008).

The observed variation in body masses in garden warblers arriving to Crete (Fig. 1) represents varia-

tion in preparation that has been done until that point. If this preparation involves accumulation, of breast muscle along with fat accumulation, the slope of the regression between breast muscle index and body mass, after the breakpoint, which in fact represents birds sampled during autumn, should be significantly different from zero, which is not the case. This implies that the preparation of muscle protein is either taking place at the breeding grounds or during the last stopover before the barrier crossing. Dietz *et al.* (2007) found that even though red knots *Calidris canutus* with high body masses close to migration departure had larger breast muscles than birds during the wintering period, the breast muscle size was, however, smaller than would be expected from an aerodynamic point of view.

It has been shown in juvenile garden warbler that they stopover for 13 to 20 days in Crete to prepare for the crossing, and during those days it is possible that a final built up of the breast muscle is taking place close to the initiation of the barrier crossing in order to cope with the extensive fuel loads needed. Unfortunately, the non-invasive technique used in this study cannot be applied on heavy birds, such as those close to departure from Crete (Fransson *et al.*, 2008), since the subcutaneous fat will cover the pectoral muscle.

In conclusion, by applying a detailed non-invasive technique for the first time in field-studies of bird migration, it is clear that a large proportion of garden warblers have undergone a large protein breakdown while crossing the ecological barrier during spring. Furthermore the lack of a clear relationship between the body mass and breast muscle index at arrival to the last stopover site before the barrier crossing indicates that the breast muscle is not built up en route from the breeding grounds to southern Europe.

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