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Usefulness of Biometrics to Analyse Some Ecological Features of Birds

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1. Introduction

Morphometric measurements of birds are the first data to be really considered as biometric in this discipline. Baldwin et al. (1931) depicted and explained in detail the external measurements used in ornithology. Currently, many of these measurements have been forgotten or are rarely used both in books dedicated to bird taxonomy (Cramp & Simmons, 1977) and in field guides on different geographical areas or on large bird groups such as shorebirds, raptors, passerines, etc. (Svensson, 1992; Baker, 1993).

Old biometric analyses used measurements performed on birds preserved in natural history museums. An appropriate representation of specimens is generally found in these museums, both in numbers (which allows for a large sample size) and in geographic origin (which enables the establishment of comparisons between birds of different areas) (Jenni & Winkler, 1989; Winker, 1993, 1996).

Body mass was another one of the data used in the initial biometric analyses. Its objective was to determine the presence of daily or seasonal variations, or variations linked to other specific periods: breeding, rearing and migration.

The next step was the establishment of a link between metric differences and the sex of birds. In some species, these differences were very visible and therefore statistical analyses were not required to support the distinction between males and females as in some raptors such as the Merlin *Falco columbarius* (Newton, 1979; Wiklund, 1990), owls and skuas (Andersson & Norberg, 1981). Similarly, marked biometric differences between bird populations of the same species found in different geographical areas were recorded (Svensson, 1992). This resulted in the identification of subspecies when these populations were geographically isolated, not sharing potential hybridization areas. Thus, for example, 10 subspecies of the Bluethroat *Luscinia svecica* have been identified throughout Europe, Asia and Alaska (Collar, 2005), a further 10 subspecies of Southern grey shrike *Lanius meridionalis* have been identified (Lefranc & Worfolk, 1997; Klassert et al., 2007), etc.

Substantial databases were created as a result of the routine collection of a minimum number of measurements when a bird was captured, this information being used for specific purposes. Possibly, the existence of these data and the ability of observation lead researchers

to conduct comparisons of measurements taken in each species, taking into account different parameters, geographical situations, habitats, etc. Thus, the first biometric studies were initiated and now days they add up to many studies already published.

Scientific articles which include morphometric measurements help to provide answers to theoretical and applied bird ecology issues (Morgan, 2004). In this chapter, we discuss how some of these issues may be analyse through biometry and which precautions need to be taken in order to avoid wrong conclusions.

2. What measures should be taken into account

Usually, wing length (maximum chord), third primary (counted in ascending order, or eighth primary counting in a descending order), tail, beak and tarsus are measured in every specimen of passerines. Most researchers follow Svensson's criteria (1992) when taking these measurements. In raptors, the measurements must be taken with a co-worker, and the following measurements are required to establish body size: a) four measurements of different parts of the right leg: tarsus-metatarsus, tibia-tarsus, middle toe and foot span, all to the nearest ± 0.1 mm; b) three measurements which include areas covered with feathers, hand-wing, the length of the right wing and the tail; c) body length (from tip of central tail feathers to crown of the bird lying relaxed on the ruler), with an accuracy of ± 1 mm. Birds moulting their longest primaries and/or the tail central feathers are excluded from the studies. Similarly, yearlings are excluded because their feathers are shorter than the adults' (Wiklund, 1996).

Body mass is usually measured with an accuracy of ± 0.1 g in small birds and ± 1 g in large birds. Two main methods are used: a) if the bird is captured and handled directly, the bird is bagged and weighed on a scale. This method can stress the bird and cause body mass reduction in a short time (Rands & Cuthill, 2001), a fact that should be taken into account when analysing data. b) When we do not wish to capture the bird, attracting it to a place situated on a scale which automatically records mass variation will suffice. This procedure has been employed, for example, to analyze body mass variation in breeding birds when they regularly visit the nest (Moreno, 1989; Szép et al., 1995), and amount of food brought to the nestlings (Reid et al., 1999), etc.

Two important issues should be taken into account in the data analysis: body mass variation with time of day and pseudoreplication of data which could distort the conclusions obtained (see review by Rands et al., 2006). Body mass shows circadian fluctuations depending on variables such as, for example, time elapsed between the time of feeding and the activity. Furthermore, there is seasonal variability depending on sex, which is more pronounced in females during the breeding period, especially in raptors (Newton, 1986).

3. Some problems with data

The quality of the measurements is essential in any scientific field but it becomes especially interesting in the study of birds. The data obtained from handling specimens (e.g., during ringing) will be subsequently analysed by other researchers, and therefore mistakes made during data collection may invalidate the rest of the work. Ensuring the quality of the measurement procedures is an essential aspect of the research.

Mentioning the quality of the measurements is equivalent to mentioning the extent of the errors. In general, the errors that can be made in this type of studies are of two types: systematic errors (bias) and random error (sampling error).

Morgan (2004) listed seven potential errors that affect the correct collection of measurements: 1) systematic *vs* random error, caused by the person taking the measurements or by the tools used; 2) errors in practice, caused by fortuitous agents at the time of taking the measurement, such as instability when measuring weight caused by the effect of the wind; 3) management error, when the measurements used for a study come from other researchers without having previously standardized the measuring protocols; 4) error from measuring devices, inaccuracy when reading the measurements of non digital equipments, as they don't always reach exactly the marks on the scales and an estimation has to be made, and each researcher can do it differently; 5) error in continuous variables, generated when the values of a continuous variable are rounded off; it must be done in accordance with the unit of measurement, as an error of 0.5 mm is not the same when measuring a passerine wing than a raptor wing; 6) errors arising from rounding off, both in continuous variables and in statistical tests in which decimal values are often rounded up or down; 7) error compounding in indices, occurring when ratios, indices, etc., are calculated by multiplying or dividing the original measurements.

The equipment used for data collection must be appropriate and must have been designed for that purpose, and the person collecting data needs to have a basic knowledge of statistical processing.

Some aspects that must be taken into account regarding the individuals who take the measurements, the repeatability of measurements taken on museum skins and on live birds, and the shrinkage effect of museum skins are discussed below.

a. The observers must be qualified for the collection of measurements as they are not the same in museum birds than in live birds and in both cases, experience and practice are required. For measurements taken on museum specimens, data to the nearest ± 0.01 mm are commonly found. For live birds, on the contrary, measurements with that accuracy are difficult to replicate, and it is therefore preferable to take measurements with an accuracy of ± 0.1 mm. To verify the error, a small sample (e.g., 10 individuals) may be taken and measurements may be repeated until appropriate handling with a minimum error is achieved.

   Whenever possible, live bird measurements should be taken by several people in order to obtain a certain range of diversification. However, an objection to this practice is the stress caused to a bird when it is handled by two or more people. On the other hand, a measurement team system (3-4 people) allows for a greater precision. This way, 1) measurements are validated when the differences obtained by each person are verified and these differences are maintained; 2) turns are taken to make the measurements so that each bird is measured by a single person but every person measures a similar number of birds; 3) if the measurements taken by each person are taken separately, the differences between them could be calculated and taken into account at the time of data analysis. In other cases, the data used for studies may come from databases from ornithological organizations, in which the data have been taken by different people but following the same measuring protocol.

b. Repeatability (known as intra-class correlation coefficient) is a statistical measurement which shows data consistency between repeated measurements of the same characteristic in a single individual. The value of the repeatability *r* is calculated using the formula $r = s^2_A / (s^2 + s^2_A)$, in which s^2_A is the value of the inter-group variance and s^2 is the value of the intra-group variance (Sokal & Rohlf, 1981). The Measurement Error (ME) which is the opposite value of repeatability and is defined as the phenotypic

proportion of a characteristic attributable to the error that may be made must also be taken into account. The value $r = 1$ is the maximum possible and shows that the measurement is completely consistent and repeatable. Measurements showing an r value below 0.70 may be considered as repeatable although, to be considered as reliable, values above 0.90 should be obtained (Harper, 1994). This calculation is important in order to ensure the accuracy of the conclusions when researchers are dependent on the collection of measurements as is the case here. Lessells & Boag (1987) indicated the existence of published and unpublished works in which repeatability had been wrongly calculated mainly as a result of applying, in the formula, the least squares values instead of the inter and intra-group variance component.

Kuczynski et al. (2003) conducted a study on Northern grey shrike *Lanius excubitor* which provided information on measurement repeatability between observers and on the differences between the measurements carried out on live birds and on birds kept in museums. Four measurements were taken (wing length, tarsus length, beak length and tail length) on 50 live specimens, their skins were prepared subsequently and the same measurements as those taken on the live birds were taken except for the tarsus because the fingers of a dissected bird's leg cannot be opened. Repeatability was calculated as intra-class correlation coefficients, and a difference in the repeatability of beak measurement was obtained. Similarly, Szulc (1964) studied three passerines (Siskin *Carduelis spinus,* Robin *Erithacus rubecula,* and Blue tit *Cyanistes caeruleus*) and found a greater variation between observers in bone measurements (beak and tarsus) than in feathers (wing and tail). Repeatability of beak length appears not to be very consistent, even when the observers are specialists in collecting these measurements, which may be due to the fact that the points from which to obtain the measurements are not well defined.

In order to avoid differences between observers, Kuczynski et al. (2003) suggested that the data should be taken by one person or by a specially trained team (see also Busse, 1983; Gosler et al., 1998). At the end of the study, Kuczynski et al. (2003) suggested the following recommendations for the collection of measurements from museum skins: 1) To define exactly the method to take the measurements required; 2) within a single study, the measurements should be taken by the same person; 3) the age of the specimen measured should be known and this datum should be included in the analysis as a covariant in order to avoid bias resulting from shrinkage.

On the other hand, Berthold & Friedrich (1979) compared two ways of measuring wing length, one based on the length of the maximum chord (Svensson, 1992) and the other based on the length of the third primary. The latter was obtained by inserting a pin mounted on a ruler between the second and third primaries near their bases, flattening the third primary and measuring it on ruler (see Bertold & Friedrich, 1979; Jenni & Winkler, 1989; Svensson, 1992). For this, the data from experienced and unexperienced observers obtained from the same 23 Tree sparrows *Passer montanus* were obtained. The mean values of the measurements taken for each one of the two groups of observers were significantly different, more so for tail length than for the length of the third primary. However, repeatability of wing length was lower than that of the third primary and therefore wing length appears to be more affected by experience or training. This is why standard ringing procedures have been regulated in England and Ireland for decades and strict training is required. As a result of this study, length of the third primary has been proposed in several countries as a measure, in passerines, which

is better than wing length to reflect body size. However, sexual dimorphism in the length of the third primary is perhaps less marked than in wing length and may therefore be a poorer measurement as sex discriminant (Gosler et al., 1995).

c. Specimen museums shrink and are dry, and therefore the length of primary feathers (and of the wing in general) is affected (Jenni & Winkler, 1989). The study of Kuczynski et al. (2003) enabled to establish the potential error resulting from shrinkage. The mean shrinkage rate between observers was different for all the measurements except for the tarsus, reaching in some cases as much as 5%. This value is above the 1 - 4 % obtained in waders and passerines (Vepsäläinen, 1968; Knox, 1980; Bjordal, 1983), although the data from these authors were obtained from a small sample size and over a short period of time following skin preparation.

On the other hand, it is common for the development of bilateral traits (e.g. wing or tarsus lengths) not to be symmetrical which pauses the issue of bilateral asymmetry, widely discussed for decades (Palmer & Strobeck, 1986). When this occurs, the issue to be resolved is which of the two tarsi or wings should be considered. In addition, it is possible that the way in which measurements are obtained by the researchers influences the values of the bilateral traits (Helm & Albretch, 2000).

4. Some applications of biometry in the study of birds

Biometry has been used to study many aspects of birds but in the present work, only four aspects will be discussed: 1) sex determination, 2) differences in size among populations, 3) wing morphology, d) body mass - body size relationship.

These sections are detailed below, including: a) the more appropriate statistical analysis in each case, b) what type of issues has the application of biometry intended to clarify, c) some specific examples of these applications.

4.1 Sex determination

Many bird species are monomorphic in their plumage and therefore sex cannot be determined through colour traits, etc. Others, on the contrary, show size differences, either of a certain trait, or of a set of traits. Thus, by determining which trait is different between sexes, it is possible to separate males from females. However, even when there are statistically significant differences in the mean values of each measurement, there is often an overlap in the measurement which renders this trait not valid as a sex differentiator (Ellrich et al., 2010).

Biometric characteristics have been used to determine the sex of birds as different as seabirds (Hansen et al., 2009), raptors (Bavoux et al., 2006), passerines (Svensson, 1992), etc. However, currently, sex can be determined using molecular techniques (Griffiths et al., 1998; Bantock et al., 2008) which are often more accurate than biometric calculations. Molecular techniques show certain disadvantages with regard to biometric techniques, among which: a) they require more time to obtain accurate results, b) they are more expensive as a well equipped laboratory is required and expensive chemical compounds are needed, c) these are invasive techniques that often require blood or feathers from live birds, although sometimes a small portion of the rachis of a feather is enough (Wang et al., 2006).

Molecular techniques have enabled to verify the validity of the biometric criteria previously used to determine sex. In general, a high level of accuracy is obtained (up to 99 %) in sex determination through biometric characteristics. However, there are also many occasions in which the error in the determination is greater than 10 % which can render the results as not

Species	Order	Sample size	Statistical analysis	Accuracy (%)	Source
Red-necked grebe *Podiceps grisegena*	Podicipediformes	76	DFA	79-80	Kloskowski et al. (2006)
Great cormorant *Phalacrocorax carbo*	Pelecaniformes	81	DFA	92-95	Liordos & Goutner (2008)
Imperial shag *Phalacrocorax atriceps*	Pelecaniformes	291	DFA	94-97	Svagelj & Quintana (2007)
Australasian gannet *Morus serrator*	Pelecaniformes	201	Two-tailed binomial test	99.5	Daniel et al. (2007)
Great egret *Ardea alba*	Ciconiiformes	79	DFA	81-88	Herring et al. (2008)
Lesser flamingo *Phoenicopterus minor*	Phoenicopteriformes	154	DFA	93-98	Childress et al. (2005)
Griffon vulture *Gyps fulvus*	Falconiformes	97	DFA	94.1	Xirouchakis & Poulakakisi (2008)
Peregrine falcon *Falco peregrinus*	Falconiformes	131 nestlings	DFA	96.2	Hurley et al. (2007)
Red-tailed hawk *Buteo jamaicensis*	Falconiformes	69	DFA	91.3	Pitzer et al. (2008)
White-tailed Eagle *Haliaetus albicilla*	Falconiformes	211 nestlings	DFA	15-98[a]	Helander et al. (2007)
Yellow-ledged gull *Larus michahellis*	Charadriiformes	155	DFA	89.5	Arizaga et al. (2008)
Black-tailed godwits *Limosa limosa*	Charadriiformes	42	DFA	95.2	Gunnarsson et al. (2006)
Black tern *Chlidonias niger*	Charadriiformes	449	DFA	81.0	Shealer & Cleary (2007)
Redshank *Tringa totanus*	Charadriiformes	157	LDF	81	Ottwall & Gunnarsson (2007)
Blue-fronted Amazon *Amazona aestiva*	Psitaciformes	202	DFA	85	Berkunsky et al. (2009)
White-throated dipper *Cinclus cinclus*	Passeriformes	231	Logistic regression	98.7	Campos et al. (2005a)

Species	Order	Sample size	Statistical analysis	Accuracy (%)	Source
Dupont's lark *Chersophilus duponti*	Passeriformes	317	DFA	99.0	Vogeli et al. (2007)
Reed bunting *Emberiza schoeniclus*	Passeriformes	99	DFA	95	Belda et al. (2009)
Corn bunting *Miliaria calandra*	Passeriformes	103	LDF	96.1	Campos et al. (2005b)
Northern great shrike *Lanius excubitor*	Passeriformes	50	LDF	85.7	Brady et al. (2009)

Table 1. Accuracy obtained in sex determination through biometric characteristics in different species, based on a sample of 20 studies published since 2005. In all cases, sex determination was also performed through molecular techniques. The type of statistical method used is detailed. LDF: Linear Discriminant Function. DFA: Discriminant Function Analysis. [a] It varied according to sex and sampling zone.

statistically valid. In a sample of 20 published studies between 2005 and 2010 (Table 1) dealing with orders as diverse as Pelecaniformes and Passeriformes, eight (40.0 %) showed an accuracy below 90 %.

It has been suggested that, whenever possible, in some species it is more advantageous to sex the two members of a breeding pair through biometric characteristics (Fletcher & Hamer, 2003). However, in passerines, this is difficult given that the overlap of the measurements is high (Gutiérrez-Corchero et al., 2007a) and, at least in Southern Europe, there are few species showing sexual dimorphism in size such as Cetti's warbler *Cettia cetti* (Bibby & Thomas, 1984) and Corn bunting (Campos et al., 2005b).

Different multivariate statistical methods are used for the classification of birds by categories. One of the most rudimentary ways of doing this is by differentiating sex based on the study of morphological traits studied separately, using bimodal distributions for their classification (Catry et al., 2005). In practice, a single variable does not provide satisfactory results, the classification being improved by the combination of more variables. In addition, it is possible for differences between groups not to be found in any of the separate variables but in their combination. On the other hand, type I error increases when conducting repeated comparisons.

The most widely used method for the determination of sex is the Discriminant Analysis. With this method, classification functions are obtained which allow to assign sex and to evaluate the quality of the results. The classifications functions are linear functions of the morphological variables considered.

In order to validate the functions, the general way of proceeding is by dividing the sample in two groups: a) the training sample, made up of data for which the sex is unmistakably known, and b) the test sample, made up of the remaining observations. When the total sample is small, the Jacknife method is frequently used. This method is part of the so-called re-sampling methods which are characterized by the fact that they hardly require assumptions on the population model from which the sample is obtained. The idea of the method, developed in various steps, consists of leaving out one datum from the observers in

each step and in calculating the classification functions using the remaining data. Once obtained, the excluded observation is classified. An analogous procedure is followed by excluding a different observation in each step.

This technique has been used in some studies (Hermosell et al., 2007). When the conditions for the application of the discriminant analysis are not met (normal distribution and identical variances) the Logistic Discriminant is used (Ellrich et al., 2010) which is based on the logistic regression. In this analysis, sex probability is estimated through a combination of explanatory variables through a logistic response model.

An issue raised recently is the variation of sexual dimorphism within and between years (Van de Pol et al., 2009), at least for some species. These authors showed that in the Eurasian oystercatcher *Haematopus ostralegus* some biometric traits used for sex determination varied through time, thus invalidating the determination of sex through biometrics. A possible solution to this problem is to calibrate these traits by month, year and area, something which seems complicated for many species.

The knowledge of the sex of each specimen favours management techniques and species conservation (McGregor & Peake, 1998). On the other hand, the knowledge of the sex of the birds studied is often essential given that individual discrimination is required in order to analyze their behaviour, etc. Spatial sexual segregation has been analysed in many bird species, mainly during the breeding season (see review of Catry et al., 2005), but also at other seasons (Campos & Martín, 2010). This raises the issue of which sex is the dominant one in each species and which habitat requirements has each sex through the annual cycle.

Another important issue which requires the prior knowledge of the sex is differential migration, understood as the variation in the distance covered and in the wintering areas according to bird categories, mainly sex and age (Ketterson & Nolan, 1983). Sex differentiation through biometric traits is very useful in this field, as during the migratory route, researchers have to handle a large number of birds in a short time.

Finally, biometry applied to sex determination enables the determination of the sex ratio in adult birds, another field which remains poorly known in spite of having been analysed for several decades (Mayr, 1939). In wild populations, there is often a bias in the proportion of sexes (see review of Donald, 2007), often in favour of males, perhaps as a result of high female mortality. Obviously, this influences population processes and, therefore, conservation of bird species.

4.2 Differences in size among populations

It is common, within a single species, for the size of the populations to vary gradually throughout their geographical distribution. The analysis of biometric differences between populations enables to relate them to environmental parameters and infer possible causes that may explain them. The study of significant differences between populations is carried out through the analysis of variance (ANOVA) on the residuals obtained from the covariance analysis models (ANCOVA) adjusted for the variables of interest in each species, including location as a factor.

Body size variation in endothermic animals has been the subject of many studies. A hypothesis put forward to explain this variation is Bergmann's rule that establishes that body size varies inversely with ambient temperature, so that body size increases with latitude, and this has been supported by some studies (Yom-Tov, 1993; Ashton, 2002; Meiri & Dayan, 2003), but not by others (Yom-Tov & Yom-Tov, 2005; Rodríguez et al., 2008; etc.).

The global warming experienced over the last decades may influence the variation in body size of birds through changes in factors such are environmental variability (Jakober & Stauber, 2000). However, there are also studies that show the difficulty of finding a relationship between global warming and body size variation (Guillemain et al., 2005; Moreno-Rueda & Rivas, 2007).

On the other hand, body size seems to be influenced by other factors apart from climatic factors such as feeding. Thus, in Blackbird *Turdus merula*, availability of food has been linked to body size increase (Yom-Tov et al., 2006) and in some passerines early nutritional stress negatively affects skeletal size that carries over into adulthood (Searcy et al., 2004).

Sometimes, biometrics also help in the taxonomy of birds as it enables subspecies differentiation. Among the various examples that could be mentioned, those of the Bluethroat, in which the subspecies *Luscinia svecica namnetum* found in France differs by its small size from others which are geographically nearby (*L. s. cyanecula* and *L. s. azuricollis*, Eybert et al., 1999), and that of the Red knot *Calidris canutus* which shows size differences between the African subspecies (*C. c. canutus*) and the subspecies from Northern Europe (*C. c. islandica*, Summers et al., 2010) are particularly clear.

The conclusions reached by applying biometric characteristics are often confirmed through genetic analyses. Currently, a greater accuracy when defining different population taxonomic categories has been achieved through the analysis of genes present in mitochondrial and/or nuclear DNA. To continue with the example of the Bluethroat, molecular genetics have confirmed the validity of the subspecies *namnetum* and also of other subspecies which are biometrically similar between them (Johnsen et al., 2006). Similarly, in the Southern grey shrike, the biometric study suggested marked differences between the subspecies *meridionalis* from the Iberian Peninsula and the subspecies *koenigi* from the Canary islands (Gutiérrez-Corchero et al., 2007a,b). The same conclusion was reached through the analysis of mitochondrial DNA, both for the cytochrome b gene (Klassert et al., 2007) and for the tandem repeats of the Control Region (Hernández et al., 2010).

Size variation is seen more clearly in large geographical areas such as a continent like Europe (Dmitrenok et al., 2007). However, it is also possible to find, within a continent, biometric differences between populations of a single species in a more reduced geographical area such as, for example, the Iberian Peninsula and the British Isles (Wyllie & Newton, 1994). This is evidenced in the White-throated dipper. Throughout Europe, its size (measured by wing and tarsus length) increases towards Northern latitudes (Esteban et al., 2000), which is in agreement with Bergmann's rule mentioned previously. However, within the Iberian Peninsula, the White-throated dippers from the South are significantly greater than those from the North (Campos et al., 2005c), which contradicts Bergmann's rule and has been explained by the influence of local environmental conditions (Arizaga et al., 2009). Therefore, biometrics also help to raise new issues on bird ecology.

Through the statistical analysis of size differences in bird populations, other issues which affect threatened species requiring special attention may be resolved. This is the case of seabirds in Northern Europe affected by human activities and dying in fishing nets or oil spills (Barrett et al., 2008). For the Common guillemot *Uria aalge,* it has been possible to determine the area from which the affected specimens came from based on body measurements, whereas in other species, this method has shown little efficacy as a result of the lack of accuracy obtained in bird size differentiation between separate colonies.

4.3 Wing morphology

The study of wing shape has been conducted, mainly, in passerines who have ten primaries in each wing. The basic data that need to be obtained are the length of each one of these feathers (the so-called primary distances) although generally, the first primary is excluded because it is very short. Generally, the fourth and fifth primary are the longest (Fig. 1) and therefore, are the ones that will define whether total wing length is larger or smaller.
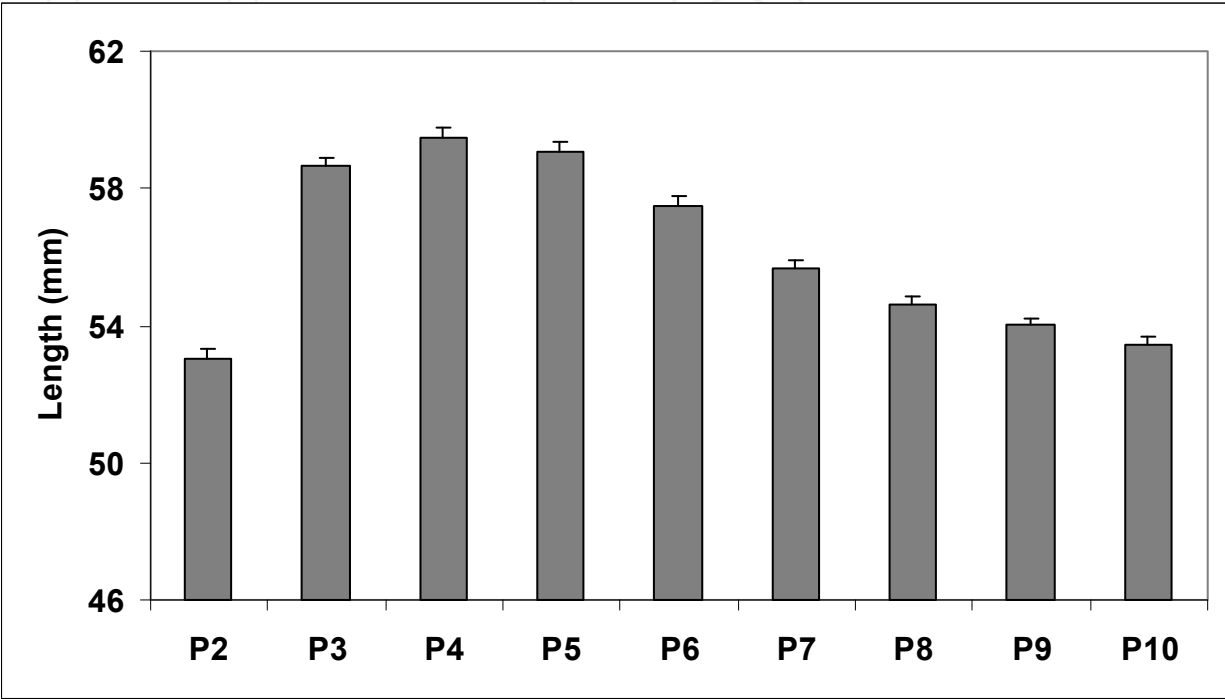

Fig. 1. Mean length (± SE) of primary feathers (P2-P10) in the wing of Bluethroat *Luscinia svecica azuricollis* in populations of central Spain.

In the majority of studies, wing morphology is characterised by the measurement of its pointedness and by its convexity, which are obtained from multivariate statistical methods. Thus, Principal Components Analysis (PCA) has been used in many studies to accurately describe the values of the primary distances using a smaller number of variables (Chandler & Mulvihill, 1988; Marchetti et al., 1995; Mönkkönen, 1995). Nevertheless, given the effect of size on wing shape, the direct application of PCA on primary distances would give wrong results. A first solution has been provided by Senar et al. (1994), who suggested a correction of the primary distances related to wing size and allometry. This method consists of multiplying the distance by a standard value of wing length divided by the specific value of bird length, raised to the power of the allometry coefficient of the distance that we wish to correct. PCA is applied on these corrected distances. The first component obtained is a good measure of wing pointedness. In spite of this correction, the results cannot be generalized either. Furthermore, this method presents statistical problems (Lockwood et al., 1998) and therefore a modification of the PCA was introduced providing a new valid method for the interpretation and characterization of the morphology within a single species and between different species (Lockwood et al., 1998). This new method is called Size-Constrained Component Analysis (SCCA). The first principal component (SCCA1) obtained through this method is a good index of wing pointedness.

Finally, the general linear model (MANCOVA) is used to study the presence of significant differences in morphological traits, controlling body size effect.

The design of bird wings is subject to various types of selective pressures. Generally, the wing is shorter and more rounded in juvenile birds than in adults (Pérez-Tris & Tellería, 2001). Longer and more pointed wings improve flight speed, whereas shorter and more rounded wings allow for better flight manoeuvrability.

Both aspects have important ecological consequences. The greater speed shortens the length of migratory journeys and therefore reduces energetic costs. Similarly, it also allows birds to reach stopover sites and wintering areas sooner, thus having an advantage over conspecifics in occupying the best sites (Bowlin, 2007, among others). On the other hand, short and rounded wings facilitate escape from predators as a result of enhanced manoeuvrability in flight, thus reducing mortality rate. Consequently, within a same species and also between species, there is a trade-off between both aspects of wing shape.

The length of primary feathers has also been analysed at the level of subspecies or migratory species populations that vary in the distance travelled in their migratory journeys. It is expected that populations travelling long distances will have longer primaries than those travelling shorter distances. This has been recorded in blackcaps (Fiedler, 2005) and bluethroats (Arizaga et al., 2006).

On the other hand, it has been detected in some non-migratory species, that some functional traits of the wings such as pointedness show covariation with weather conditions and the structure of the habitat they occupy (Vanhooydonck et al., 2009). This may be important to show the speed at which bird adaptations take place in changing local conditions.

All these questions require a knowledge of wing shape, for which biometrics are essential. Nevertheless, over the last years, it has become quite common to analyse the migratory behaviour of many bird species through stable hydrogen isotopes present in the feathers (Hobson, 2005). That way, the place of origin of the birds captured may be determined more accurately during their migratory flights or in the wintering areas. However, this method is laborious and expensive, and in addition it requires the extraction of one or several feathers from the bird. As for sex determination, when the handling of a large number of birds is required, the help of biometric analyses has shown to be important to resolve ecological issues related to migratory birds, given that it is simple, quick and its cost is low.

4.4 Body size – body mass relationship

Frequently, in birds, the greater the body size, the greater the body mass. The size of body mass may reflect the nutritional status of the bird (and therefore its fitness) and hence it is necessary to know its value.

Variation of birds' body condition is a subject of great interest in evolutionary ecology, and an accurate knowledge of it enables to confirm theories on bird adaptations to different environmental conditions. Thus, for example, the starvation-predation risk trade-off theory predicts that, in birds, body mass increases when starvation risk is greater and decreases when predation risk increases (McNamara & Houston, 1990; MacLeod et al., 2008). It is known that birds carry fewer fat reserves than the maximum possible (Witter & Cuthill, 1993), perhaps because body mass reduction favours greater flight manoeuvrability (Witter et al., 1994) and therefore, preys can escape more easily from predators, reducing thus predation risk (Lima, 1986; McNamara & Houston, 1990; Cresswell, 1998; MacLeod et al., 2005). For predatory birds, a greater manoeuvrability in flight may facilitate the capture of prey. On the other hand, body mass increase favours the resistance to adverse

environmental conditions and to food unpredictability, especially when birds must face a reduction in prey numbers.

There are many ways of analysing body condition in birds (refer to the review by Brown, 1996): size of subcutaneous fat reserves (Redfern et al., 2000), haematocrit (Cuervo et al., 2007), blood albumin level (Ardia, 2006), etc., but a simple one is the relationship between body size (generally expressed as wing or tarsus length) and body mass.

Body mass - body size relationship must be statistically analysed in order to ensure that the conclusions reached are accurate. Generally, a comparison of body mass in different groups is conducted, correcting the potential existing differences between them as a result of size that could affect the results. The statistical methods used for this are:

1. Ratio Index. It is the simplest and is calculated by dividing body mass by a measurement of size, for example tarsus or wing length, or by some power of it (Albrecht et al., 1993). This index has been criticized as a result of the problems it presents (Jacob et al., 1996). Atchely et al. (1976) showed that the ratio variables are skewed to the right, leptokurtic and that the non-normality is increased when the denominator coefficient is increased. Further, multivariate statistical procedures are affected when the analyses include ratios. And what is worst, it has been proved that in the scaling of data, ratios do not remove the effect of the scaling variables.

2. Residual Index (RI). This procedure is based on the least squares linear regression of body mass over size. Once the regression has been conducted, the residuals obtained are considered as a measure of body condition. In most studies, a single measure of body size is usually used to perform the regression. Given that the objective is to eliminate the effect of body size, a possibility for obtaining greater accuracy could be to perform Principal Component Analysis between different body measurements (e.g., tarsus or wing length) and conduct the regression with this new variable. In spite of being one of the methods which are used most frequently, the comparisons between RI values are not always valid. Furthermore, it has been shown that, often, the required hypotheses for the use of the least squares residuals are not met, and thus the errors of the test hypothesis increase. The use of the reduced major axis regression is therefore more appropriate (Green, 2001).

3. Analysis of Covariance (ANCOVA). This is a statistical control technique which is used to isolate the effect of a variable. It has the advantage of integrating in a single procedure the regression analysis and the analysis of variance procedures. Some authors recommend the use of this method exclusively in order to eliminate the effect of the value of body mass (García-Berthou, 2001).

An example based on the Southern grey shrike shows the different conclusions reached using one method or another. The Southern grey shrike is a medium size bird (25 cm) whose sexes remain separate during the non-breeding period: males remain in the breeding territories and females occupy distant areas (Campos & Martín, 2010). Campos et al. (2008) analysed the seasonal variation in the relationship body size - body mass in agricultural areas of Northern Spain, separating males and females. For this, they used the residual index RI calculated on the body mass - tarsus length regression. Their conclusion was that during the non-breeding season, the RI value did not vary significantly between autumn (October and November) and winter (December to February), and neither did they vary significantly between sexes or within each sex.

In the present chapter, unpublished data to date on this variation in Southern grey shrike in the centre of Spain where the environmental conditions in the study area are similar to those

in Campos et al. (2008) are presented. The ANCOVA procedure was used to compare the relationship between body mass and body size for different season, sex, age (yearling or adult) and habitat (irrigation crops *vs* non-irrigated crops), each of them with two levels. The prototypic analytic model for these outcomes was a four-way ANCOVA using tarsus (indicator of body size) as covariate. Main effects and interactions that were not significant at P > 0.05 were removed so that the best model could be fitted to the data. Additionally two-way ANCOVA models were used to assess differences in mean between groups examined by the independent variables of season, habitat and sex, respectively.

The full and adjusted 4-way ANCOVA models for the body mass were significant (Table 2). Because sex was not significant either in the main effect or in the interaction effect, this variable was removed in the following analysis. In the adjusted model the two significant effects found were the main effect of season (P < 0.001) and the habitat x season x age interaction (P = 0.026).

When examined by habitat, the significant effect in this analysis was the main effect of season for both, non-irrigated crops and irrigated crops (P = 0.029 and P < 0.001, respectively, Table 3).

	Full factorial model				Adjusted model		
	df	F	P		df	F	P
Main effects							
Habitat	1	1.717	0.192		1	0.476	0.491
Season	1	11.054	0.001		1	15.886	<0.001
Age	1	2.064	0.152		--	--	--
Sex	1	0.068	0.795		1	0.067	0.795
Two-way interactions							
Habitat x Season	1	0.890	0.347		1	2.673	0.104
Habitat x Sex	1	0.370	0.544		--	--	--
Habitat x Age	1	0.606	0.437		1	1.275	0.261
Season x Sex	1	0.142	0.707		--	--	--
Season x Age	1	0.529	0.468		1	0.026	0.872
Sex x Age	1	0.489	0.485		--	--	--
Three-way interactions							
Habitat x Season x Sex	1	0.024	0.876		--	--	--
Habitat x Season x Age	1	4.431	**0.037**		1	5.004	**0.026**
Habitat x Sex x Age	1	0.239	0.626		--	--	--
Season x Sex x Age	1	0.504	0.478		--	--	--
Four-way interactions							
Habitat x Season x Sex x Age	1	0.820	0.366		--	--	--
Covariates							
Tarsus	1	22.820	<0.001		1	28.511	<0.001
Overall model	17	6589.764	<0.001		9	11776.667	<0.001

Table 2. Full and adjusted ANCOVA models taking into account body mass, body size, habitat, season, age and sex. Statistically significant interactions are in bold. In the adjusted model, no significant main and interaction effects (P > 0.05) were removed if they were not included in higher order interactions. df: degree of freedom. P: probability.

	Non-irrigated crops				Irrigated crops		
	df	F	P		df	F	P
Main effects							
Season	1	4.903	0.029		1	22.989	<0.001
Age	1	0.170	0.681		1	1.282	0.260
Two-way interactions							
Season x Age	1	0.873	0.352		1	2.145	0.146
Covariates							
Tarsus	1	20.152	<0.001		1	21.835	<0.001
Overall model	5	10586.661	<0.001		5	12193.111	<0.001

Table 3. Variables that influence body mass in accordance to habitat. df: degree of freedom. P: probability.

Significant differences in body mass of the shrikes in autumn and in winter were recorded in both types of crops (Table 4 by rows).

	Autumn	Winter	Difference
Non-irrigated crops	64.65 ± 2.79 (11)	63.88 ± 2.51 (55)	0.77
Irrigated crops	65.35 ± 2.67 (32)	61.76 ± 3.23 (20)	3.59

Table 4. Mean value ± SD of body mass in adult Southern grey shrikes according to habitat (non-irrigated crops, irrigated crops) and season (autumn, winter). Sample size in brackets. The difference between mean values is adjusted.

Furthermore, non significant main effects or interactions were found for autumn, but habitat was significant (P = 0.045) for winter (Table 5).

	Autumn				Winter		
	df	F	P		df	F	P
Main effects							
Habitat	1	0.045	0.832		1	4.099	0.045
Age	1	0.494	0.484		1	0.564	0.454
Two-way interactions							
Habitat x Age	1	1.603	0.209		1	1.182	0.279
Covariates							
Tarsus	1	19.229	<0.001		1	23.105	<0.001
Overall model	5	10455.906	<0.001		5	12478.849	<0.001

Table 5. Variables that influence body mass according to season. df: degree of freedom. P: probability.

Indeed, the mean value of body mass of the shrikes was greater in the birds captured in non-irrigated crops than in birds captured in irrigated crops (Table 4, by rows).

Finally, when examined by age, the main effect of season was significant for both, yearling and adult (P = 0.05 and P < 0.001, respectively, Table 6) and the interaction habitat x season was significant for adult (P = 0.029). Body mass mean value (± SD) varied significantly

between autumn and winter both in young shrikes (64.1 ± 3.1 N = 48 *vs* 62.2 ± 3.5, N = 57) and in adults (65.1 ± 2.6, N = 43 *vs* 63.3 ± 2.8, N = 75).

	Yearling				Adult		
	df	F	P		df	F	P
Main effects							
Habitat	1	1.608	0.208		1	0.883	0.349
Season	1	8.322	0.005		1	14.428	<0.001
Two-way interactions							
Habitat x Season	1	0.116	0.735		1	4.907	0.029
Covariates							
Tarsus	1	33.048	<0.001		1	10.384	0.002
Overall model	5	9506.195	<0.001		5	14127.559	<0.001

Table 6. Variables that influence body mass according to age. df: degree of freedom. P: probability.

The body mass - body size relationship has also been used to analyse other ecological issues in birds such as offspring quality. The measurements obtained on nestlings are a good example to analyse bilateral assymetry and to verify which factors have an influence on body development of their bilateral traits. In this case, the issue to be resolved is which tarsus or wing must be related to body mass.

5. Further research

It can be inferred from the paragraphs detailed above that the following issues should be analysed in more detail in future research on:

a. The way in which to increase the accuracy of measurements, unifying measurement criteria until their use becomes universal. This will enable the comparison of data obtained from different researchers and will facilitate reaching valid conclusions in studies based on animals from different geographic origin.

b. Sex determination from biometric traits so that accuracy is close to 100%. That way bird sex may be determined through simple, quick and cheap methods. The importance of knowing the sex of a bird in a wide type of ecological studies has been shown above.

c. Variation of biometric characteristics of birds according to their distribution area also requires further studies. Variables which allow to accurately determine, for example, where the birds captured in a study area come from are required. This aspect appears to be essential in order to analyse the behaviour of migratory species.

d. Biometric traits – body size relationship until an almost perfect adjustment is obtained. New biometric characteristics which so far have been poorly explored and that would enable a more accurate statistical adjustment should be studied. An example of this has been the use of the third primary of the wing (see paragraph 3) instead of the total length (maximum chord).

e. Similarly, the body size – body mass relationship should be further studied until the most suitable biometric characteristics are found in order to analyse them statistically. To that effect, it would be convenient to detail what type of mathematical analyses should be applied in each type of study, so that their use can be generalized and comparable results may be obtained in any part of the world.

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Edited by Dr. Midori Albert

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