ESTIMATION OF THE PROTEIN RESERVES OF WADERS : THE USE AND MISUSE OF STANDARD MUSCLE VOLUME

by Theunis Piersma , Nick Davidson and Peter Evans

Many workers have examined the body condition of birds at various stages of their annual cycle. Both fat and protein reserves must be measured in studies of this kind, if the body condition of a bird is to be described adequately, since the two have different functions (see e.g. Evans and Smith 1975, Davidson 1981a, b). Although techniques for assessing the fat and protein reserves of live birds exist (for waders see e.g. Davidson 1979, 1983, Pienkowski, Lloyd and Minton 1979, McNeil and Cadieux 1972), accurate measurement of body condition in individual birds has proved possible so far only from laboratory analysis of carcasses (Davidson 1983, 1984a).

Techniques for estimating fat reserves, using organic solvents such as petroleum ether or chloroform and methanol, are widely used (Kerr, Ankney and Millar 1982). Since almost all the fat extracted by these techniques is available as a reserve (only a small percentage being structural), the size of the fat reserve is taken usually as the mass of fat extracted (e.g. Marcstrom and Mascher 1979, Marcstrom and Kenward 1981, Davidson and Evans 1982, Piersma 1984). Fat reserves are often expressed as an index, usually the mass of fat as a proportion of total mass or lean dry mass. Such indices are largely independent of body size.

The largest protein reserves are stored in the musculature (Kendall, Ward and Bacchus 1973). They are difficult to measure, since by no means all the muscle protein is available for use during starvation (Davidson and Evans 1982, Piersma 1984). The reserve cannot, therefore, be measured by analysing the total protein content of the muscle, although the two measures are certainly correlated. Moreover, such total protein estimations are very time-consuming. However, since changes in muscle mass generally reflect changes in the size of the protein reserve (Kendall, Ward and Bacchus 1973), lean dry muscle size provides an index of the protein rserves. Since in practice it is very difficult to remove all the musculature from a carcass, the protein reserve has been measured usually as an index calculated from the size of the largest muscle block(s). In many birds, including waders, these are the pectoral muscles, which make up over half the total musculature. It should be noted that this is not always the case: in many aquatic birds including geese, ducks, grebes and auks (Hanson 1962, pers. obs.), leg muscle mass may equal or exceed pectoral muscle mass and so must be used in addition to the pectoral muscle mass to derive the protein index.

Lean dry muscle mass varies with body size as well as with the size of the protein reserve. Thus direct use of lean dry muscle mass as an index can be very misleading in both intra- and interspecific comparisons, particularly where individual variation is being studied. Lean dry muscle mass has, however, been used widely in studies of the protein condition of wildfowl populations (*e.g.* Hanson 1962, Ankney 1977). Expressing muscle mass as a proportion of total lean, or lean dry, mass minimises the effects of body size variation (Evans and Smith 1975, Davidson 1981a,b, 1984b, Piersma 1984). However, since a change in muscle mass accompanied by the same proportional change in total lean (or lean dry) mass gives an unchanged index, the use of this type of index is limited also.

An alternative way of minimising the effects of body size variation in comparisons of muscle mass is to use skeletal measures of the muscle attachment to give a standardised index. Thus Schifferli (1976) and Jones (1980) standardised the pectoral muscle mass of House Sparrows *Passer domesticus* by using the slope of the relationship between the diagonal measure of the muscle attachment (from the posterior point of the keel to the distal point of the coracoid bone) and the lean dry mass of the pectoral muscles. Since the mass of the muscles should be proportional to its volume, the relationship with a single (length) measure of muscle attachment should not be linear, so the slope should vary.

To exclude the effects of body size variation in Bar-tailed Godwits *Limosa lapponica* Evans and Smith (1975) derived a Standard Muscle Volume (SMV) from 4 dimensions of the skeletal attachment of one pectoral muscle block. Their index of protein reserve size was the lean dry mass of the pectoral muscles (pectoralis major and supracoracoideus) of one side of the body, as a proportion of the SMV calculated for that bird. Such an index is dimensionless. This index has been used subsequently in several studies of the body condition of other wader species, notably by workers at Durham and Groningen Universities (e.g. Davidson 1980, 1981a,b, 1982, 1984b, Davidson and Evans 1982, Kersten and Piersma 1983).

We discovered recently that the figure in Evans and Smith (1975) showing the skeletal measurements for calculation of the standard muscle volume had misled at least one worker. Incorrect measurement in that case (documented below) led to considerable error in the indices of protein reserves that resulted. This paper aims to clarify the application of the technique, so as to avoid any possible future confusion. In addition we take the opportunity to give the derivation of the SMV formula for waders: this derivation was not given in Evans and Smith's original (1975) paper. By modifying this derivation, appropriate formulae may be derived for birds with different sternum morphology to waders. It must be stressed that the formula derived below, and given in Evans and Smith (1975), is valid only for waders, and appropriate constants must be found for other groups of birds. Such work is in progress.

CORRECT AND INCORRECT STERNUM MEASUREMENTS

The original drawing in Evans and Smith (1975) of a generalised wader sternum showing the 4 skeletal measurements used to calculate SMV is reproduced in Figure 1a. Here, and elsewhere, a = the 'internal' length of the sternum at the junction of the bony raft and the keel, b = the maximum height of the keel of the sternum, measured perpendicular to a at the anterior point of measurement of a, c = the distance from the anterior point of a to the distal end of the coracoid bone, and d = the width of the

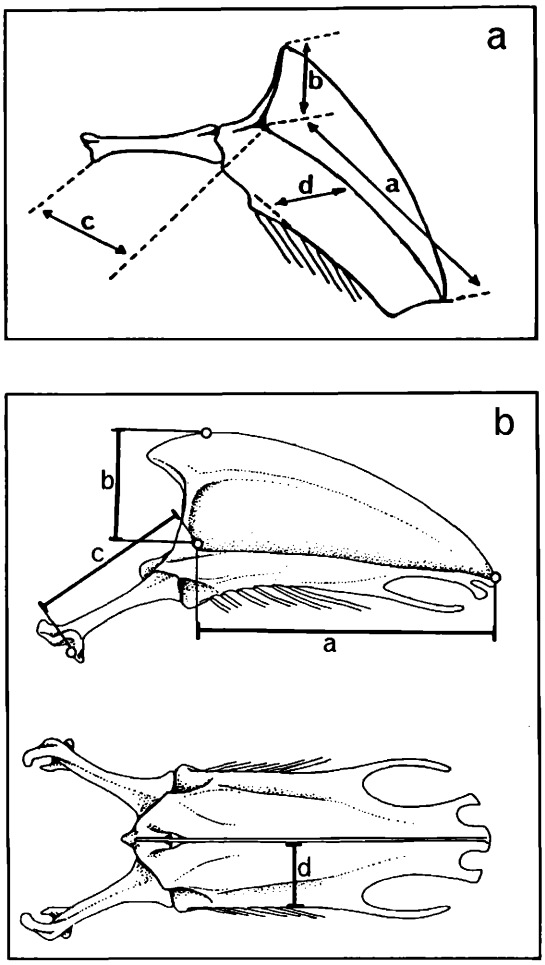

Figure 1. The skeletal measurements of the sternum and coracoid used to calculate Standard Muscle Volume, from equation [9]. a) original Figure 1 from Evans and Smith (1975) of a generalised wader sternum; b) lateral and ventral views of the sternum of a Redshank *Tringa totanus*.

bony raft of the sternum. The more detailed lateral and ventral view of the sternum of a Redshank *Tringa totanus* (Figure 1b) also show the correct positions of these measurements. In Figure 1b we show the position of measurement *d* at the most posterior rib attachment to the raft of the sternum. This differs slightly from the position of *d* shown by Evans and Smith (see Figure 1a), but makes the position of *d* easier to replicate whilst not altering its size. The measurements should be made with dividers or fine-pointed calipers. For values comparable with those in Evans and Smith (1975), all measurements should be in cm.

One analyst measuring waders in the Netherlands had interpreted Evans and Smith's figure (Figure 1a) incorrectly. His interpretations of measurements *a* – *d* are shown in Figure 2b, alongside the correct locations for the measurements (Figure 2a). Table 1 compares the measurements of Oystercatchers *Haematopus ostralegus* measured in this incorrect manner, with a sample of Oystercatchers of similar body size measured correctly by one of us (Th. P.). We were unfortunately unable to measure the same carcasses for the comparison in Table 1. Average body size (measured by bill-length and wing-length) was very similar in the two samples (Table 1). However, as would be expected from comparison of the positions of measurements in Figure 1a and b, the average values of *a* – *d* differ, especially *a* (too long) and *c* (too short). Although the deviations from the correctly measured values tend to cancel out, the values of SMV calculated from the incorrect measurements were still on average 5% larger than the correctly measured values. Thus the incorrect measurements resulted in underestimation of the size of the protein reserves.

DERIVATION OF THE SMV FORMULA PUBLISHED IN EVANS AND SMITH (1975)

P.R.E. was responsible for inventing a standard muscle shape for waders from examination of the pectoral muscles and their attachment in several species. This shape is shown schematically in Figure 3. Assuming a

Table 1. Comparison of the lengths of skeletal measurements a – d of the sternum and coracoid of Oystercatchers measured correctly (Figure 2a) and incorrectly (Figure 2b), and wing-lengths and bill-lengths of the samples. Note that the measurements used to calculate SMV were made on 2 different samples. Means ± S.D. are given. All measurements are in cm.

	correct measurement (Figure 2a)		incorrect measurement (Figure 2b)	
	males	females	males	females
n	15	14	30	54
a	5.84 ± 0.15	5.73 ± 0.21	7.29 ± 0.26	7.23 ± 0.24
b	2.37 ± 0.13	2.27 ± 0.16	2.57 ± 0.17	2.64 ± 0.20
c	4.10 ± 0.23	4.08 ± 0.18	3.01 ± 0.14	2.98 ± 0.15
d	1.84 ± 0.09	1.86 ± 0.14	1.85 ± 0.12	1.92 ± 0.08
SMV	4.28 ± 0.41	4.07 ± 0.49	4.48 ± 0.40	4.47 ± 0.44
wing	25.8 ± 0.90	25.8 ± 0.80	25.7 ± 0.70	26.0 ± 0.80
bill	7.01 ± 0.24	7.62 ± 0.51	7.04 ± 0.38	7.92 ± 0.39

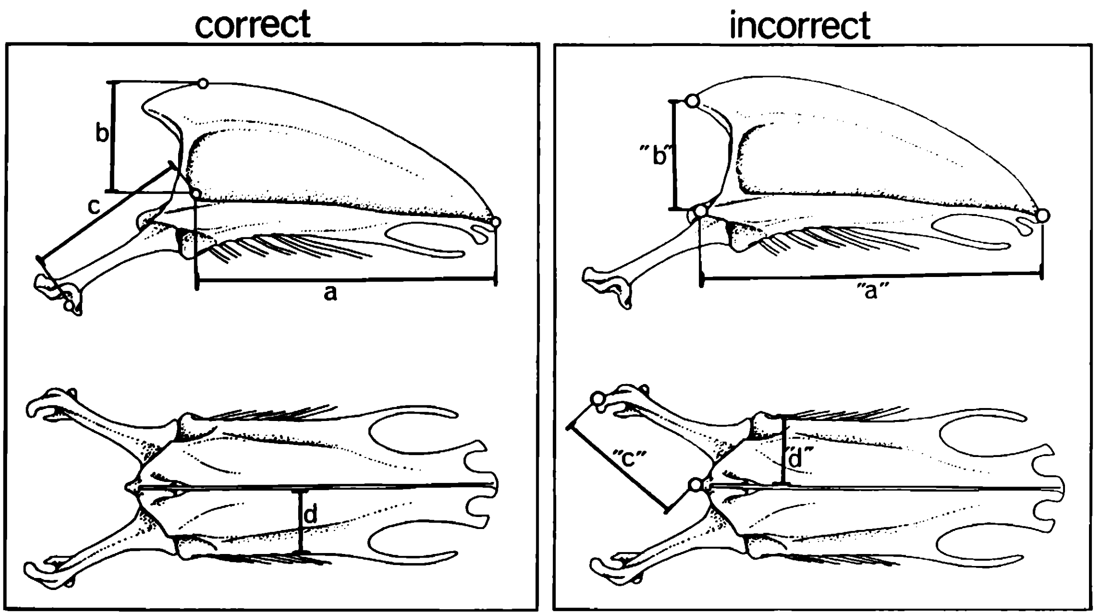

Figure 2. Correct and incorrect interpretations of Evans and Smith's original (1975) Figure 1 (see text). Lateral and ventral views of the sternum of a Redshank are shown, from Figure 1*b*.

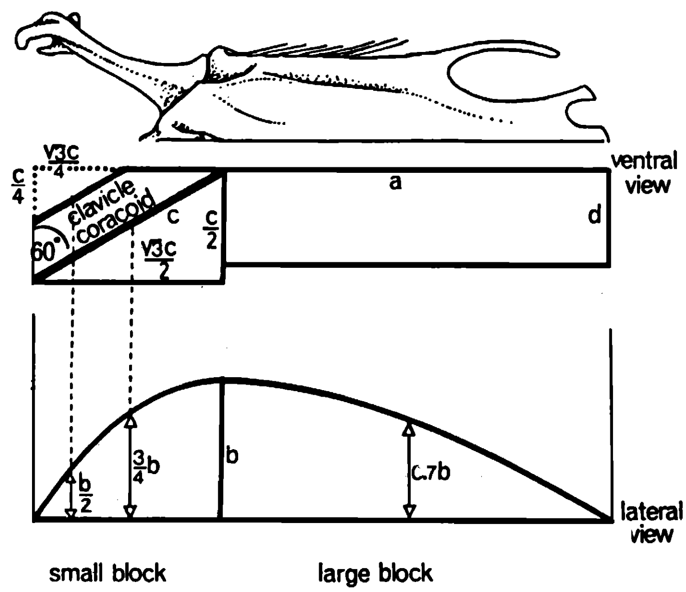

Figure 3. Schematic diagram of the sternum of a Redshank, showing the derivation of the formula for SMV. See text for further explanation of the derivation.

trapezoid muscle shape (the fine detail of the shape does not matter for an index such as SMV), the pectoral muscles of one side of the body can be divided into 2 blocks. These are a small block attached to the coracoid bone, and a large block along the sternum (Figure 3).

In addition to measurements *a* – *d*, three other measurements need to be taken for derivation of the formula for the volume of the small block. These are:

e) the angle between the coracoid bone and a line perpendicular to the mid-line of the bird;
f) the position of the proximal end of the clavicle, as a proportion of the mid-line distance between the proximal and distal points of *c*; and
g) the position of the distal end of the clavicle, as a proportion of the perpendicular distance from the mid-line to the distal point of *c*.

For waders, $e = 60°$, $f = 1/2$, and $g = 1/2$.

From Figure 3, the dorsal area of attachment of the small block, including the dotted outline, would be :

$$\frac{\sqrt{3}c}{2} \times \frac{c}{2} \qquad [1]$$

and the average depth of attachment 3b/4, so that the volume would be:

$$\frac{\sqrt{3}c^2}{4} \times \frac{3b}{4} \qquad [2]$$

However, the volume of the hollow between the fused clavicles (wishbone) must be deducted. The cross-section area of this is:

$$\frac{1}{2} \times \frac{\sqrt{3}c}{4} \times \frac{c}{4} \qquad [3]$$

and the average depth is *b*/2 (see Figure 3). Hence the volume to be subtracted is:

$$\frac{\sqrt{3}c^2}{4} \times \frac{b}{16} \qquad [4]$$

Thus the volume of the small block is:

$$\frac{\sqrt{3}c^2}{4}\left(\frac{3b}{4} - \frac{b}{16}\right) = \frac{11b}{16}\left(\frac{\sqrt{3}c^2}{4}\right) \qquad [5]$$

$$= (0.69b) \times (0.433c^2)$$

$$\hat{=} (0.7b) \times (0.433c^2) \qquad [6]$$

From Figure 3, the dorsal area of attachment of the large block is a x d, and the average depth of attachment is 0.7b. Thus the volume of the large block is:

$$0.7b \times a \times d \qquad [7]$$

The SMV is the sum of the volumes of the large block and the small block:

$$(0.7b) \times (0.433c^2) + (0.7b \times a \times d) \qquad [8]$$

$$= 0.7b(0.433c^2 + ad) \qquad [9]$$

which is proportional to

$$b(0.433c^2 + ad) \qquad [10]$$

This SMV formula applies to waders (Charadrii) only. Differences in the shape of the sternum and pectoral muscle block of other groups of birds mean that constants in the formula will differ from those for waders. A separate formula should be derived to calculate SMV for these other groups of birds.

WHY USE SMV?

A major advantage of using SMV to measure pectoral muscle size and protein reserves is that it provides an index that is theoretically independent of body size. Intraspecific independence from body size can be shown by comparing correlations of body size and muscle mass with correlations of body size and SM Index. For example the lean dry mass of the pectoral muscle of a single sample of adult Dunlins *Calidris alpina* from North Wales increased with body size (measured by total head length) ($n = 32$, $b = 0.028 \pm 0.0072$, $F = 14.9$, $P < 0.001$). In the same sample, however, SM Index was unrelated to total head length ($b = -0.001 \pm 0.0015$, $F = 0.15$, $P > 0.10$). Similar relationships exist for other body size measures (e.g. bill-length and wing-length), areas and species. A detailed study of how SMV and SM Index vary with body size will be published elsewhere (Davidson and Piersma in prep.). Without such independence from body size, detailed comparison of protein reserves between birds of different size is difficult, because of the problems of scaling. Provided that the morphology of the sternum and coracoid bones are similar, both intra- and interspecific comparisons can be made. The ability to make interspecific comparisons is a particular advantage of SMV over other indices of muscle size. Use of SMV has shown, for example, that plovers (Charadriidae) have larger protein reserves than sandpipers (Scolopacidae). This difference holds both for small species of similar size, for example Ringed Plover *Charadrius hiaticula*, Dunlin *Calidris alpina* and Sanderling *C. alba*, and for larger species such as Grey Plover *Pluvialis squatarola*, Golden Plover *P. apricaria*, Knot *Calidris canutus* and Bar-tailed Godwit (Davidson 1981a,b).

We have described the use of SMV to give an index of protein reserves, but do variations in pectoral muscle size really reflect differences in protein reserve size? Brittas and Marcstrom (1982) found that lean dry pectoral muscle mass was correlated closely with the total protein mass in the muscle. Furthermore, Kendall *et al.* (1973) showed that at least some changes in pectoral muscle mass were the result of changes in the protein reserve. Recent evidence for waders (N.C. Davidson and P.R. Evans unpubl.) has shown that the SM Index is indeed a good correlate of the size of the labile protein reserves in the pectoral muscles of several small wader species.

ACKNOWLEDGEMENTS

Frans Kerkum (Rijkdienst voor de Ysselmeerpolders) most generously allowed us to use his measurements of Oystercatchers as a warning to others. Without his work, the problems in interpreting the original description of the SMV would not have come to our attention. Piet Zegers, Leo Zwarts and Jan Hulscher helped in sorting out the Oystercatcher data. The Redshank sternum was drawn carefully by Ruurd Noordhuis, and Steve Carter redrew a figure. Marcel Kersten, Mike Pienkowski, Kees Rappoldt and Dave Townshend criticised earlier drafts of this note most enthusiastically.

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