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Flight style in bats as predicted from wing morphometry: the effects of specimen preservation

O. R. P. BININDA-EMONDS AND A. P. RUSSELL

Vertebrate Morphology Research Group, Department of Biological Sciences, The University of Calgary, 2500 University Drive N.W., Calgary, Alberta, Canada T2N 1N4

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(With 5 figures in the text)

An as yet unconsidered potential error in studies that predict flight style from morphological measurements of bats is the effect of the specimen type employed. On the basis of the finding that morphological measurements taken from fluid-preserved bat specimens may not yield values equivalent to those taken from the live animal, we compared the values of several variables (lifting surface area, wingspan, mass, aspect ratio, wing loading and minimum power speed) for live and fluid-preserved little brown bats (*Myotis lucifugus*) with the accepted standards for this species given by Norberg & Rayner (1987). Significant differences were detected for lifting surface area, wingspan, mass, aspect ratio and wing loading values taken from live bats and their respective values reported by Norberg & Rayner. Differences between preserved bats and Norberg & Rayner's numbers were limited to lifting surface area and wingspan (extended wing positions only), aspect ratio (all wing positions), and mass (both 70% ethanol- and 45% isopropyl alcohol-preserved specimens). Thus, Norberg & Rayner's values correspond most closely to values obtained from preserved museum specimens, a fact reflecting the source of their data in this instance. This and other limitations involved in attempting to predict the flight style of bats from a few morphological characters are discussed.

Contents

Introduction

Since flight is the primary means of locomotion of bats, an accurate description of a species' flight performance will yield valuable information about its ecology. However, the secretive and nocturnal habits of bats make it difficult to observe their flight styles directly. Researchers have therefore attempted to predict flight performance on the basis of a few key measurements or morphological indices obtained from the bat wing: wingspan, wing area, wing loading and aspect ratio (e.g. Poole, 1936; Findley, Studier & Wilson, 1972; Lawlor, 1973; Norberg, 1981; Aldridge, 1986; Norberg & Rayner, 1987). In the most comprehensive study of this kind, Norberg & Rayner (1987) predicted the likely flight behaviour of all previously studied bat species, primarily upon the basis of their aspect ratio and wing loading. In so doing, Norberg & Rayner necessarily relied heavily on literature sources for raw data for many taxa.

A potential shortcoming of such studies is that the data (and the ecological conclusions derived from them) are dependent upon the source specimens. In Norberg & Rayner's paper, live animals (e.g. Jones, 1967; Jones & Suttkus, 1971), freshly killed specimens (e.g. Hartman, 1963; Lawlor, 1973), and museum specimens, both study skins (e.g. Farney & Fleharty, 1969) and alcohol-preserved specimens (e.g. Vaughan, 1959, 1966), were used as sources of raw data, and these different sources were often pooled together for a given bat species. However, we (Bininda-Emonds & Russell, 1993) have subsequently found that the representative specimen types for bats (live animal, freshly killed specimen and fluid-preserved specimen) should not be directly compared with one another since the values obtained for the key measurements outlined above often differ significantly between the different types. Therefore, a question equal in importance to 'What flight behaviour can a given species of bat exhibit?' is, 'Does our perception of what this flight behaviour might be change according to the type of specimen we are examining?'

The purpose of this study, then, was to investigate the second question above: do our perceptions actually change? To accomplish our goal, 26 individuals of a single species of bat, the little brown bat (*Myotis lucifugus*), were fixed and preserved according to standard museum techniques. The values for all the variables examined (lifting surface area, wingspan, mass, aspect ratio, wing loading and minimum power speed) were compared to their respective reported values presented by Norberg & Rayner (1987). We then compared the different specimen types to other vespertilionid species of a similar flight style (as evidenced by similar values for aspect ratio and wing loading) and to the values of *Myotis lucifugus* reported by Norberg & Rayner (1987). A point of major significance here is that the same set of individuals yielded the data for all specimen types, thus allowing changes induced during the preservation procedure to be directly monitored.

We hasten to add that this paper is not intended as an indictment of Norberg & Rayner's (1987) approach or conclusions, but is meant to point out some of the limitations of all such studies that attempt to characterize the flight styles of bats by using morphological variables. Norberg & Rayner's contribution is merely the most comprehensive and most recent of these kinds of study and their numbers are often taken as the standard by which to measure one's own data (e.g. Aldridge, 1988).

Methods

A detailed description of specimen collection and preparation, and of the tracing and measuring protocols, can be found in Bininda-Emonds & Russell (1993). Four different specimen types were examined, which represent the sequential stages in the preservation process for fluid-preserved museum specimens: live animals, freshly killed specimens, specimens immediately after fixation and rinsing ('post-fixation') and specimens that have been preserved in alcohol for some time (36-week-preserved specimens). All of these specimen types, with the exception of the post-fixation stage, are routinely used as data sources for bat specimens. Within the post-fixation and 36-week-preserved samples the wings were fixed in 1 of 3 positions: 'compressed' bats are those preserved with the wings folded against the body, 'intermediate' bats are those preserved with the wings partially spread (so that the individual digits are recognizable), and 'extended' (Bininda-Emonds & Russell, 1993) bats are those preserved with 1 wing (here, the left) fully extended and the other fully compressed. Although the intermediate and extended positions are rarely found in museum collections, we found them to be demonstrably better than the compressed position in preserving the original wing morphometry (Bininda-Emonds & Russell, 1993).

Morphological measurements, indices and flight speeds

Lifting surface area (LSA; analogous to the more equivocal term 'wing area') was defined as in Norberg (1981) (see Bininda-Emonds & Russell, 1993: fig. 1). Wingspan was defined as in Bininda-Emonds & Russell (1993).

Aspect ratio (A), wing loading (Q_s), and minimum power speed (V_{mp}) were calculated as noted in Bininda-Emonds & Russell (1993: 147, equations (1), (2), and (3), respectively). Aspect ratio (A) describes the relative antero-posterior width of the wing (i.e. narrow versus broad) (Findley *et al.*, 1972), and, as such, is a good indicator of the general shape of the wing (Aldridge, 1986). Wing loading (Q_s) describes the body weight supported per unit area of the flight surface (McManus & Nellis, 1972) and correlates with minimum flight, minimum power, and maximum range speeds, turning radius, and general manoeuvrability of a given species (Pennycuick, 1975; Aldridge, 1986; Norberg, 1987). Minimum power speed (V_{mp}) is used to maximize flight time for a given amount of energy (Norberg & Rayner, 1987). The body mass from the freshly killed specimen was used to calculate both wing loading and minimum power speed for all stages.

Aspect ratio and wing loading categories

Norberg & Rayner (1987) based their predictions of a given species' flight performance on its aspect ratio and wing loading, assigning most species to a qualitative category for each variable (generally low, average, or high). No explicit account was given of how membership in a particular category was determined for a given species. Norberg & Rayner (1987: 381–382) state that 'aspect ratio and wing loading refer both to the absolute values of these quantities... and to the size-independent measures of these quantities derived from (a) principal components analysis.' Wing loading was further characterized relative to the size of the bat (as represented by mass). We question this latter technique as the mass of the bat is already incorporated into the wing loading. By scaling wing loading relative to mass, one runs the risk of inadvertently creating false trends as the 2 variables are necessarily correlated with each other (autocorrelation). As there was no overall size estimate of the bat available in Norberg & Rayner (1987) that was independent of LSA, we simply judged wing loading according to its absolute magnitude alone.

In order to view the distribution of these categories for vespertilionids (the family to which *Myotis lucifugus* belongs), we plotted histograms of the number of vespertilionid species of a given numerical aspect ratio (or wing loading) according to the category they were assigned by Norberg & Rayner. The boundaries between the categories were quantified by sorting all the aspect ratio (or wing loading) values in ascending order and dividing this series sequentially into categories. The boundaries were set so that each category contained the same number of species as did the original category in Norberg & Rayner's paper (1987). (The 'average to low' wing loading category mentioned by Norberg & Rayner (1987: 397) for vespertilionids of low aspect ratio is obviously an artificial clustering of bats of average and low wing loading in that one instance. In determining the boundaries for the wing loading categories, we assumed that this 'category' contained equal numbers of bats of average and of low wing loadings.)

Statistical analysis

The initial hypothesis for this study was that there would be no differences between the values of any of the 6 variables (LSA, wingspan, mass, aspect ratio, wing loading and minimum power speed) determined at a given stage in our sample and the equivalent values presented by Norberg & Rayner (1987). Only the results for the live animals and the 36-week-preserved specimens were compared to Norberg & Rayner's values by way of a 2-tailed one sample Student's t-test using Systat 5·0 on an IBM PC (Zar, 1984; Wilkinson, 1990). These comparisons were made according to treatment groupings found in our earlier study (Bininda-Emonds & Russell, 1993); live bats were all pooled together, and 36-week-preserved specimens were pooled according to wing position for all variables except for mass, which was pooled according to preservation fluid. A rejection level of 0·05 was used, corrected for multiple comparisons with the Bonferroni method (Eq. 1) (Snedecor & Cochran, 1989):

$$\alpha_C = \alpha_E / r \quad (1)$$

where α_C = rejection level for multiple comparisons; $\alpha_E = 0{\cdot}05$; r = number of comparisons.

Smooth curves for the histograms were generated by using Systat's KERNEL smoothing algorithm (Wilkinson, 1990). The graph of aspect ratio versus wing loading was created by plotting the individual data points and determining 95% Gaussian bivariate confidence ellipses to view the range of these 2 variables for a number of cases (Wilkinson, 1990). Three sets of ellipses were plotted. The first was for each of the 4 preservation stages. Although we earlier found significant differences between the wing positions in each preservation stage for aspect ratio and wing loading (Bininda-Emonds & Russell, 1993), the wing positions were pooled for each stage to simplify the graph. Additionally, ellipses were plotted for all specimens grouped over all preservation stages (all stages) and for all specimens of all preservation stages except the post-fixation stage (all stages less post-fixation). The exclusion of the one stage in the latter pooled ellipse reflects the artificial nature of the post-fixation stage.

TABLE I

Values of a given variable for a given specimen and the respective value presented by Norberg & Rayner (1987). Values presented in parentheses represent the corrected values of those variables from the sources used by Norberg & Rayner (1987). The 36-week-preserved specimens are compared according to (a) fixation wing position or (b) preservation fluid. Numbers are presented as mean ± S.E. except for Norberg & Rayner (1987) which are presented as means only

(a)

Variable[1]	Norberg & Rayner (1987)	Live all bats (n = 26)	36-week-preserved specimens: Compressed (n = 9)	Intermediate (n = 9)	Extended (n = 8)
LSA	0·0093 (0·0086)	0·0113 ± 0·00013	0·0096 ± 0·00037	0·0101 ± 0·00033	0·0107 ± 0·00023
B	0·237	0·252 ± 0·0012	0·239 ± 0·0047	0·238 ± 0·0038	0·251 ± 0·0025
A	6·0 (6·3)	5·6 ± 0·049	6·0 ± 0·058	5·6 ± 0·041	5·9 ± 0·048
Q_s	7·5 (8·0)	6·9 ± 0·187	7·7 ± 0·252	7·9 ± 0·228	7·6 ± 0·187
V_{mp}	3·248 (3·286)	3·205 ± 0·034	3·288 ± 0·051	3·378 ± 0·040	3·300 ± 0·028

(b)

Variable[1]	Norberg & Rayner (1987)	Live all bats (n = 26)	36-week-preserved specimens: 70% ethanol (n = 14)	45% isopropyl alcohol (n = 12)
M	0·071	0·085 ± 0·0026	0·091 ± 0·0044	0·114 ± 0·0022

[1] LSA: Lifting surface area; B: wingspan; M: mass; A: aspect ratio; Q_s: wing loading; V_{mp}: minimum power speed

Results

We noted an apparent error in the value for LSA given for *Myotis lucifugus* by Norberg & Rayner (1987). On the basis of their original sources, we calculated that their value for LSA should have been 0·0086 m^2, not 0·0093 m^2 as reported. This lower value for LSA causes Norberg & Rayner's values for aspect ratio, wing loading and minimum power speed to increase to 6·3, 8·0 N/m^2 and 3·286 m/s, respectively (Table I).

Values for four of the six variables (LSA, wingspan, mass and aspect ratio) determined from our sample of live animals were significantly different from those published by Norberg & Rayner (1987) (Table I). Additionally, at 36 weeks of preservation time, LSA and wingspan for the extended bats, aspect ratio for the intermediate bats, and mass for bats preserved in both ethanol and alcohol differed significantly between our sample and their respective values from Norberg & Rayner (1987). Comparisons with the corrected values did not change this pattern greatly,

TABLE II

t values from a two-tailed one-sample Student's t-test comparing values of a given variable for a given specimen type with the respective value presented by Norberg & Rayner (1987). Comparisons were made between (a, b) the actual value published in Norberg & Rayner (1987) or (c) the corrected value determined from the original sources used by Norberg & Rayner (if applicable); see Table I. The 36-week-preserved specimens were compared according to (a, c) fixation wing position (b) preservation fluid

(a)

Variable[1]	Live all bats ($n = 26$)	36 week-preserved specimens: Compressed ($n = 9$)	Intermediate ($n = 9$)	Extended ($n = 8$)
LSA	16·10*	0·72	2·36	6·05*
B	12·72*	0·34	0·30	5·86*
A	−7·47*	−0·48	−8·53*	−1·36
Q_s	−3·16	0·63	1·67	0·68
V_{mp}	−1·26	0·78	3·24	1·83

(b)

Variable[1]	Live all bats ($n = 26$)	36-week-preserved specimens: 70% ethanol ($n = 14$)	45% isopropyl alcohol ($n = 12$)
M	5·35*	4·54*	19·03*

(c)

Variable[1]	Live all bats ($n = 26$)	36-week-preserved specimens: Compressed ($n = 9$)	Intermediate ($n = 9$)	Extended ($n = 8$)
LSA	21·70*	2·63	4·52	9·17*
A	−14·36*	−6·30*	−16·74*	−8·51*
Q_s	−5·74*	−1·28	−0·45	−1·90
V_{mp}	−1·26	0·03	2·29	0·49

*, $P < 0·05$ (adjusted for multiple comparisons)
[1] See Table I for explanation

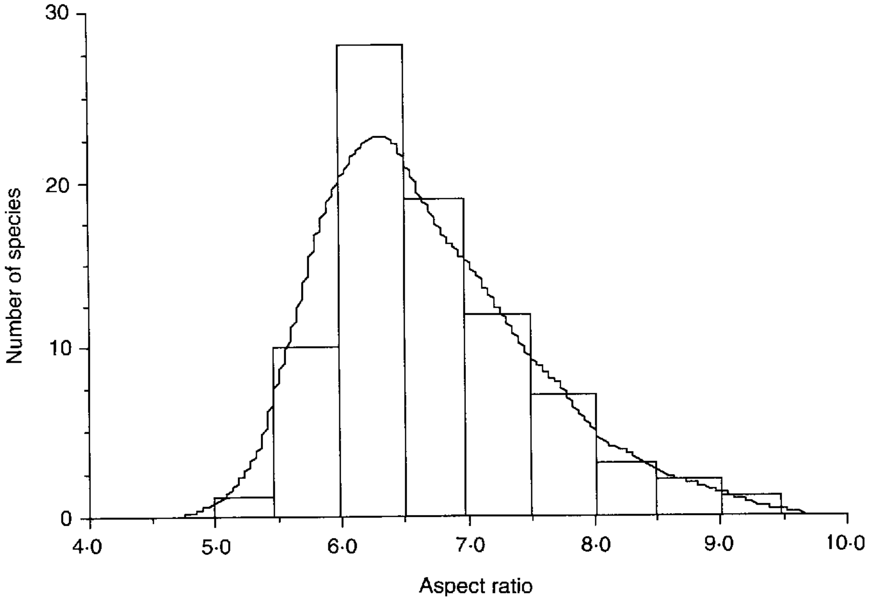

FIG. 1. Histogram of the number of vespertilionid species of a given aspect ratio. Only those species assigned a qualitative category by Norberg & Rayner (1987) are included (see Fig. 2).

producing only a few additional differences to those already found. Wing loading in the live animals was now found to be significantly smaller than the corrected Norberg & Rayner (1987) value. Also, our values for aspect ratio in all preserved bats (regardless of wing position) were significantly smaller than the corrected value from Norberg & Rayner. Only minimum power speed never differed significantly between our sample and either its respective or corrected value from Norberg & Rayner (1987) (Tables I and II).

The vespertilionids (or at least that portion of the family that was placed into the qualitative categories by Norberg & Rayner (1987)) span a wide range of both aspect ratio and wing loading values (Figs 1 and 3). However, the categories employed by Norberg & Rayner do not subdivide the respective continua with any apparent utility (Figs 2 and 4). The categories for each index overlap greatly and do not display any obvious discontinuities. This is especially true for wing loading, where the mode for all categories except for high is at about 7·0 N/m^2. As might be expected, the clumped 'average to low' wing loading category displayed a bimodal distribution, although the distribution of the average category completely encompassed that of the low category. Our method estimated the boundaries for the aspect ratio categories to be as follows: low, less than 6·1; average, 6·1–7·3; and high, greater than 7·3. Likewise, the boundaries for wing loading categories were estimated to be as follows: very low, $< 6{\cdot}45\ N/m^2$; low, 6·45–7·5 N/m^2; average, 7·5–10·3 N/m^2, and high, $> 10{\cdot}3\ N/m^2$.

A plot of aspect ratio versus wing loading demonstrated clear differences among the different specimen types for *M. lucifugus* employed in this study (Fig. 5a). Only the 95% confidence ellipses for the post-fixation and 36-week-preserved bats overlap, and then only to a small degree. The ellipse representing the freshly killed specimens is reasonably well removed from the ellipses of the remaining specimen types. Both of the two pooled ellipses are centred around an aspect