MINIMIZATION OF POTENTIAL PROBLEMS ASSOCIATED WITH THE MORPHOMETRY OF SPIRIT-PRESERVED BAT WINGS

OLAF R. P. BININDA-EMONDS AND ANTHONY P. RUSSELL

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

Abstract.—An important but largely ignored problem in using museum specimens of bats in morphometric studies is that changes are induced in the specimens during the preservation process. Values obtained from preserved specimens may thus differ markedly from those obtained from the living animal. A brief diagnosis of this problem in dealing with chiropteran specimens is presented, as is a summary of the current knowledge dealing with potential changes during preservation of study skins and alcoholic chiropteran specimens. Finally, it is suggested that a standardized procedure for obtaining wing tracings be used by specimen collectors, museums, and chiropteran researchers to alleviate or at least minimize these problems for bat specimens.

An assessment of the lifting surface area (Fig. 1), usually simply referred to by the misleading term "wing area," of bat species forms a vital component of many chiropteran studies. With a knowledge of the value of lifting surface area (LSA), it is possible to predict a large portion of the flight performance of a particular bat species by examining its flight efficiency (derived from aspect ratio) and minimum flight, minimum power, and maximum range speeds, turning radius, and general manoeuverability (derived from wing loading) (Pennycuick, 1975; Aldridge, 1987; Norberg, 1987). The accuracy of such predictions, however, depends upon the degree to which the measurement of LSA from the specimen reflects the actual value possessed by the living animal.

Heretofore, discussion involving LSA determination has centered primarily on which portions of the bat's anatomy should be included in the LSA, or what technique of determining the LSA yields the most accurate results. However, underscoring this discussion is a very basic problem; do changes occur to the LSA as a result of the various preservation techniques used to produce permanent specimens from which such measurements can be taken? The seriousness of this problem is thrown into sharp focus when it is realized just how many chiropteran studies have used preserved museum specimens to obtain raw data for subsequent analysis (e.g., Vaughan, 1959, 1966; Struhsaker, 1961; Jones, 1967; Farney and Fleharty, 1969; Findley *et al.*, 1972; Myers, 1978; Smith and Starrett, 1979; Norberg, 1981; Baagøe, 1987; Norberg and Rayner, 1987). This list is by no means exhaustive.

Common preservation modes for bat specimens are the preparation of study or flat skins and fluid-preserved specimens (DeBlase and Martin, 1981). Thus far, no studies have specifically examined changes induced (if any) in LSA as a result of preservation. There are, however, indications that changes might occur.

It has been noted that forearm lengths decrease in dried and skinned bat specimens due to desiccation and compaction of the wrist elements (Arata, 1968). Such drying out and compaction probably occur elsewhere in bat study skins, especially in the wing membrane. The magnitude of this potential decrease in

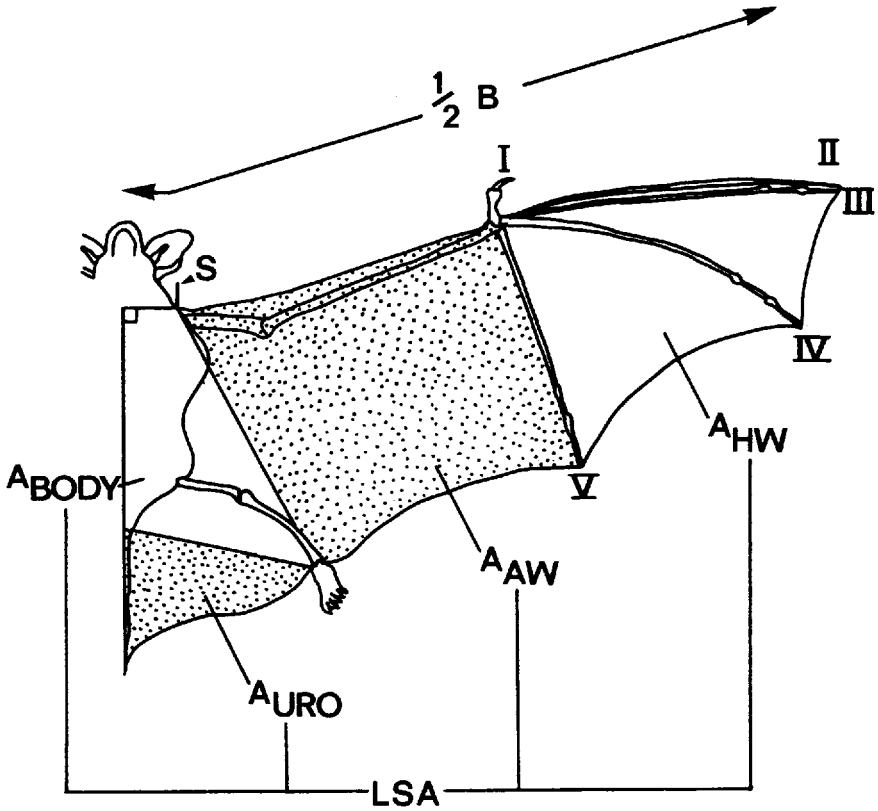

Figure 1. Diagrammatic representation of *M. lucifugus* showing definition of one-half of the wingspan (½ B), and boundaries of one-half of the lifting surface area (LSA) and its subunits: handwing area (A_{HW}), armwing area (A_{AW}), body area (A_{BODY}), and uropatagial area (A_{URO}). LSA does not include the area of the head. Digits are indicated by Roman numerals. S marks the position of the left shoulder. (Adapted from Saunders, 1989).

wing membrane area has not been investigated to date. Furthermore, it is difficult to carry out morphometric work on bat study skins because of the risk of damaging the wings. Ideally, one should only make wing tracings from bat study skins where at least one wing has been preserved in an "extended" position, i.e., spread out (Hangay and Dingley, 1985; Blood and McFarland, 1988). This extended position is not generally recommended, however, as it requires considerable space for storage and the specimens are also more susceptible to breakage (Wagstaffe and Fidler, 1968). Such factors limit the amount of usable bat study skin material available to researchers interested in wing morphometry and its role in the understanding of bat flight. Flat skins present the additional problem of converting a three-dimensional animal to essentially two dimensions. Thus, the body area calculated from such specimens will be larger than that recorded from equivalent (three-dimensional) study skins—an unwanted and undetermined source of variation.

Bats are also routinely stored as fluid-preserved specimens to avoid the desiccation of the wings (Rosevear, 1965). Although such specimens provide in-

valuable data for research on bats, our investigations (Bininda-Emonds and Russell, in press) indicate that care must be exercised when using them for inferring aspects of flight morphology or performance. Preparation of such specimens involves a two-step procedure: fixation, typically in 10% neutral buffered formalin, and preservation, in 65–70% ethanol, 45–60% isopropyl alcohol, or 10% neutral buffered formalin (Nagorsen and Peterson, 1980; DeBlase and Martin, 1981). We were able to standardize preparation and preservation conditions for a series of bats of known provenance and living dimensions, and to conduct a protracted series of observations on them throughout the preservation process. This has revealed potentially serious problems resulting from changing specimen dimensions in employing such specimens for morphometric studies of the flight performance of bats (Bininda-Emonds and Russell, in press).

Both LSA and wingspan of little brown bat (*Myotis lucifugus*) specimens were found to be dependent upon the specimen type examined during the fluid-preservation process. Bininda-Emonds and Russell (in press) documents measurement and assessment procedures, and preservation effects. Complicating this basic difference between specimens were differences between the preserved specimens depending upon the fixation position of the wings ("compressed," "intermediate," or "extended"). Although originally suggested for study skins and not fluid-preserved specimens (Wagstaffe and Fidler, 1968; Hangay and Dingley, 1985), the intermediate and extended wing positions were demonstrably better than the conventional compressed position. These results were primarily evident when LSA was determined by tracing the preserved bat specimens onto paper and digitizing the outlines to determine their areas.

These "tracing procedures" are of generally limited use for preserved specimens due to the potential effects of formalin fixation on the collagen network of the wing membranes (Holbrook and Odland, 1978; Viidik 1980). Some procedures that estimate LSA based on idealizing the wing as a combination of simple geometric figures were initially proposed with the hope that they would be immune to the effects of formalin as they rely on measurements of the forearm and various digits of the wing. Unfortunately, this generally has not proved to be the case. These "estimation procedures" are similarly affected by the fixation and preservation procedure, i.e., results are dependent on the specimen type examined and, for preserved specimens, on the fixation position of the wing. The procedures of Pirlot (1977) and Blood and McFarland (1988) significantly underestimated LSA for all specimen types. Only Smith and Starrett's (1979) procedure yielded accurate estimates of LSA for live specimens and for preserved specimens with the wings fixed in the extended position (Bininda-Emonds and Russell, in press).

Due to their nocturnal and secretive nature, it is difficult to directly observe most bats in the wild. Thus, bat researchers rely on predicting the flight behaviour of a bat species (a key factor determining the ecology of these animals) from its morphology. However, we have shown that we may be seriously misrepresenting what a given bat species is capable of in the field based on measurements obtained from fluid-preserved specimens (Bininda-Emonds and Russell, in press). It is vital, then, to find a procedure that will let us accurately assess LSA and the other morphometric characters used to predict flight behaviour.

One simple solution for minimizing the potential errors outlined above when using fluid-preserved specimens is to use only specimens where one of the wings

has been fixed in the extended position. This is the only wing position that will yield accurate LSA values for fluid-preserved specimens (with respect to the live animal) whether through tracing procedures or Smith and Starrett's (1979) estimation procedure. This suggestion is not likely to be followed, however, because of the impracticality of the extended wing position (Wagstaffe and Fidler, 1968). Fixing bats in the extended position in the field would be problematic for many collectors, and the extended specimens require more storage space, a valuable commodity in most museums.

An even better solution would be to use the estimation procedure provided by Aldridge (1988) which uses mass to estimate LSA. No significant differences were found between LSA estimated from live mass and the actual traced live LSA (Bininda-Emonds and Russell, in press). Thus, as long as the preserved specimen has had its live mass recorded, an accurate assessment of LSA can be made. However, a drawback of this and any other estimation procedure is that they accurately estimate only one definition of LSA and, more importantly, they do not provide any estimates of the areas of the various subunits comprising the LSA. A solution that yields more general output is thus required.

Museums already compile a number of characteristic measurements for bat specimens: forearm length, tragus length, and occasionally wingspan (Nagorsen and Peterson, 1980). We suggest the compromise solution that a wing tracing of the live or freshly-killed specimen also be taken. Many museums already retain catalogues, field notes, photographs, and maps of collecting sites pertaining to individual specimens (Nagorsen and Peterson, 1980), so the addition of a wing tracing should not be an unreasonable demand. As demonstrated by Saunders (1989), wing tracings can be performed in the field and so should not present a serious problem to collectors who begin preparation procedures in such situations.

An accurate value for the LSA is certainly as important to chiropteran studies today as are the other measurements noted above. The advantages to be gained from having such information available more than compensate for the inconvenience incurred in making the tracing. The area of the wing tracing need not be calculated by the collector or museum either, but merely stored for future reference. Access to the wing tracing would allow researchers with different definitions of the LSA and its subunits to follow their own procedures and to minimize variability in their samples, while avoiding the inaccuracy that comes with recording wing dimensions from preserved specimens. All that need be done is to follow a specific set of instructions for making the tracing and to ensure that demarcation points are marked on the tracing to enable the areas of appropriate subunits of the LSA to be measured (Fig. 1).

The exact procedure for obtaining this wing tracing should be standardized and formalized to minimize error between collectors. We suggest the following simple method. The live or freshly-killed bat should be placed on a sheet of paper on its back to minimize rolling and to keep the wing as flat to the paper as possible. The left wing should then be stretched to its fullest extent without damaging it, pulling the leading edge of the wing to lie as nearly perpendicular as possible to the long axis of the body. The left hind limb and the uropatagium should also be stretched out and held flat. Small weights may be employed to aid in keeping the wing membranes flush with the paper. The position of the shoulders (the points at which the wings insert into the body) should then be marked (to demarcate the

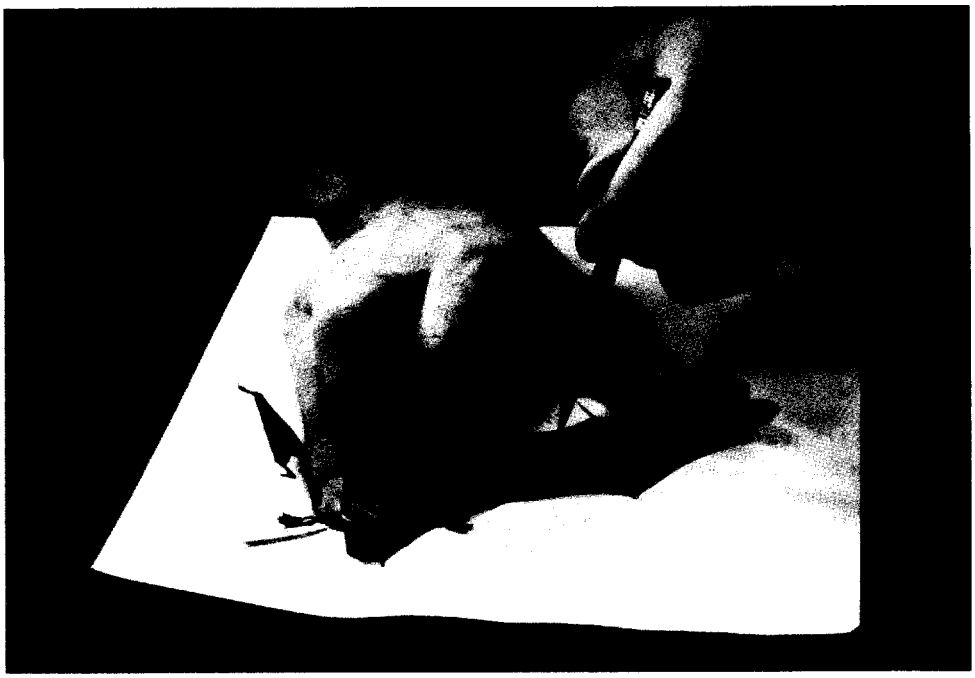

Figure 2. Photograph illustrating our suggested technique for tracing the left wing of a bat specimen. Note the small brass weights holding down parts of the patagium.

boundary between the wing and the body), followed by an outline extending around the left wing and uropatagium to the tail tip (Fig. 2). Such raw data, if cross-referenced to voucher specimens, can then be maintained in a file until such time that they are required for analysis.

Acknowledgments

We thank Mr. Larry Powell and two anonymous reviewers for providing valuable comments on an earlier version of this manuscript. This work was made possible by an NSERC summer studentship to Olaf Bininda-Emonds and an NSERC operating grant (No. 0GP0009745) to Anthony P. Russell.

Literature Cited

Aldridge, H. D. J. N. 1987. Turning flight of bats. Journal of Experimental Biology, 128:419–425.

———. 1988. Flight kinematics and energetics in the little brown bat, *Myotis lucifugus* (Chiroptera: Vespertilionidae), with reference to the influence of the ground effect. Journal of Zoology, 216: 507–517.

Arata, A. 1968. Discrepancy between "dry" and "fresh" forearm measurements in bats. Journal of Mammalogy, 49:155–156.

Baagøe, H. J. 1987. The Scandinavian bat fauna: Adaptive wing morphology and free flight in the field. Pp. 57–74 in *Recent Advances in the Study of Bats* (M. B. Fenton, P. A. Racey, and J. M. V. Rayner, eds.). Cambridge University Press, Cambridge, 470 pp.

Bininda-Emonds, O. R. P., and A. P. Russell. In press. Effects of preservation on wing morphometry of bats: A test case based on the little brown bat (*Myotis lucifugus*). Journal of Zoology.

Blood, B. R., and D. A. McFarland. 1988. A new method for calculating the wing area of bats. Mammalia, 52:600–603.

DeBlase, A. F., and Martin, R. E. 1981. *A Manual of Mammalogy, 2nd edition.* Wm. C. Brown Company Publishers, Dubuque, Iowa, 436 pp.

Farney, J., and E. Fleharty. 1969. Aspect ratio, loading, wing span and membrane area of bats. Journal of Mammalogy, 50:362–367.

Findley, J. S., E. H. Studier, and D. E. Wilson. 1972. Morphologic properties of bat wings. Journal of Mammalogy, 53:429–444.

Hangay, G., and M. Dingley. 1985. *Biological Museum Methods.* Academic Press, Sydney, 379 pp.

Holbrook, K. A., and G. F. Odland. 1978. A collagen and elastic network in the wing of the bat. Journal of Anatomy, 126:21–36.

Jones, C. 1967. Growth, development, and wing loading in the evening bat, *Nycticeius humeralis* (Rafinesque). Journal of Mammalogy, 48:1–19.

Myers, P. 1978. Sexual dimorphism in size of vespertilionid bats. American Naturalist, 112:701–712.

Nagorsen, D. W., and R. L. Peterson. 1980. *Mammal Collectors' Manual: A Guide for Collecting, Documenting and Preparing Mammal Specimens for Scientific Research.* Life Sciences Misc. Publ., Royal Ontario Museum, Toronto, 79 pp.

Norberg, U. M. 1981. Allometry of bat wings and legs and comparison with bird wings. Philosophical Transactions of the Royal Society London, B 292:359–398.

Norberg, U. M. 1987. Wing form and flight mode in bats. Pp. 43–56 in *Recent Advances in the Study of Bats* (M. B. Fenton, P. A. Racey, and J. M. V. Rayner, eds.). Cambridge University Press, Cambridge, 470 pp.

Norberg, U. M., and J. M. V. Rayner. 1987. Ecological morphology and flight in bats (Mammalia; Chiroptera): wing adaptations, flight performance, foraging strategy and echolocation. Philosophical Transactions of the Royal Society London, B 316:335–427.

Pennycuick, C. J. 1975. Mechanics of flight. Pp. 1–75 in *Avian Biology, Volume V* (D. S. Farner, J. R. King, and K. C. Parkes, eds.). Academic Press, New York, 523 pp.

Pirlot, P. 1977. Wing design and the origin of bats. Pp. 375–410 in *Major Patterns in Vertebrate Evolution* (M. K. Hecht, P. C. Goody, and B. M. Hecht, eds.). Plenum Press, New York, 908 pp.

Rosevear, D. R. 1965. *The Bats of West Africa.* Trustees of the British Museum (Natural History), London, xvii + 418 pp.

Saunders, M. 1989. Resource partitioning between little brown bats (*Myotis lucifugus*) and long-legged bats (*Myotis volans*) in southern Alberta. MSc Thesis, University of Calgary, Calgary, 138 pp.

Smith, J. D., and Starrett, A. 1979. Morphometric analysis of chiropteran wings. Pp. 229–316 in *Biology of Bats of the New World Family Phyllostomatidae, Part III* (R. J. Baker, J. K. Jones, Jr. and D. C. Carter, eds.). Special Publication 16, Museum, Texas Tech University, Lubbock, Texas, 441 pp.

Struhsaker, T. T. 1961. Morphological factors regulating flight in bats. Journal of Mammalogy, 42: 152–159.

Vaughan, T. A. 1959. Functional morphology of three bats: *Eumops, Myotis, Macrotus.* University of Kansas Publication, Museum of Natural History, 12:1–153.

Vaughan, T. A. 1966. Morphology and flight characteristics of molossid bats. Journal of Mammalogy, 47:249–260.

Viidik, A. 1980. Interdependence between structure and function in collagenous tissues. Pp. 257–280 in *Biology of Collagen* (A. Viidik and J. Vuust, eds.). Academic Press, New York, 384 pp.

Wagstaffe, R., and J. H . Fidler. 1968. *The Preservation of Natural History Specimens, Volume II.* H. F. & G. Witherby Ltd., London, 404 pp.