*J. Zool., Lond.* (1993) **230,** 141–158

# Effects of preservation on wing morphometry of the little brown bat (*Myotis lucifugus*)

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*

(*Accepted 15 March 1992*)

(With 3 figures in the text)

The effects of formalin fixation and subsequent alcohol preservation on various morphometric variables and their derivatives (lifting surface area, wingspan, mass, aspect ratio, wing loading and minimum power speed) and on the results of procedures that estimate lifting surface area of the little brown bat (*Myotis lucifugus*) are identified and quantified. Statistical analysis demonstrates that the values of all of the examined morphometric variables depends upon the specimen type from which they are obtained (live animal; freshly killed specimen; immediately following formalin fixation; or after 36 weeks in alcohol). Over the short term, the choice of preservation fluid is not important with respect to determination of the six variables studied. The fixation position of the wing is an important factor in the determination of all variables except mass. Although originally suggested for study skins and not fluid-preserved specimens, 'intermediate' and 'extended' wing positions are demonstrably better than the conventional 'compressed' position. The estimation procedures of both Pirlot (1977) and Blood & McFarlane (1988) significantly underestimate analogous lifting surface areas determined by tracing live bats. Smith & Starrett's (1979) procedure was found to yield accurate estimates occasionally: on live animals and preserved specimens with wings fixed in the extended position. Aldridge's (1988) method also yields accurate estimates of lifting surface area, but is limited to those museum specimens where the live or freshly-killed mass is known. Such conclusions permit recommendation of procedures that minimize changes arising through the fixation and preservation process in fluid-preserved museum specimens when compared to the live animal.

## Contents

## Introduction

Among mammals, bats are distinguished by their morphology associated with powered flight. The almost exclusive reliance on flight as a means of locomotion enables a large part of the basic ecology of these animals (e.g. habitat types exploited during foraging) to be predicted through a description of the limits of their flight performance. Up until now, this has been achieved by deducing flight performance from a few key measurements or morphological indices obtained from the wing: wingspan, wing area, wing loading, aspect ratio and wingtip indices (e.g. Poole, 1936; Struhsaker, 1961; Findley, Studier & Wilson, 1972; Lawlor, 1973; Norberg, 1981; Aldridge, 1986; Norberg & Rayner, 1987).

Morphological data are often obtained from museum specimens such as study skins or alcohol-preserved specimens (e.g. Struhsaker, 1961; Farney & Fleharty, 1969; Findley *et al.*, 1972; Smith & Starrett, 1979). While the preparation of study skins and skulls is the norm for small mammals, bats are atypical in that they are often preserved as alcoholic specimens to prevent drying out of the wings (Rosevear, 1965). The limitations of using fluid-preserved specimens for obtaining morphometric data have been documented for anurans (Lee, 1982) and especially for fish (e.g. Jones & Geen, 1977), but only alluded to for bats (Vaughan, 1966; Myers, 1978).

The effects of preservation on representatives of the 'lower' vertebrate classes are numerous, often quite unpredictable, and are reasonably well known because specimens of these groups are routinely preserved and stored in liquid media. Measured characters respond differentially to preservation, with some increasing in size, others decreasing, and a few others showing reversals (e.g. initially increasing in size and then decreasing or *vice versa*) (Jones & Geen, 1977; Lee, 1982). Integumentary features were shown by Lee (1982) to be particularly prone to the effects of preservation. Distortions that have been documented are also size independent, meaning that any distortion is proportionately greater for smaller individuals of a given species (Lee, 1982).

Following Lee's (1982) findings for anurans, we decided to test for the possibility of similar effects of preservation on bats. It is apparent that data obtained from fluid-preserved bat specimens may differ from those gleaned from the live animal, but the extent of these differences remain unexplored. Given the importance attached to the data obtained from morphometric measurements of bat wings, such assessments become particularly important for these animals.

The network of bundles of collagen and elastic fibres found in the bat wing (Holbrook & Odland, 1978) are likely to deform and denature with exposure to formalin. Such effects have been demonstrated for other collagenous tissue (Viidik, 1980). In addition, Viidik (1980) found that formalin fixation increases the stress–strain curve of collagenous tissue (rat skin) fixed under tension, thus potentially altering its response to future stresses (such as stretching).

Myers (1978) suggested that differences apparent in various types of alcoholic specimens may be due to the choice and concentration of the preservation fluid. The pH of the preservation media may also be important. Viidik (1980) has shown that the tensile strength, elastic stiffness, and failure energy of rat tail tendons decrease with decreasing pH. Standard preservation fluids are 45%–60% isopropyl alcohol (more properly known as 2-propanol) and 65%–70% ethanol (Nagorsen & Peterson, 1980). If these preservation media differ in pH, a different response of the collagen network in the bat wing may occur between the two fluids; it should be easier to stretch the

wings, and hence obtain a larger wing area value, for bats placed in the more acidic alcohol. Alcohols are also known to oxidize with age and thus decrease in pH (Korcek *et al.*, 1972). This might result in age-dependent differences with older specimens being easier to stretch, again resulting in larger wing area values.

Furthermore, Viidik (1980) has shown that differential deformation of the collagen network occurs in rat skin depending on the tension under which the skin is placed while it is being fixed. Three commonly suggested wing positions for bat study skins are: 'compressed', with the wings completely folded and pressed against the body (Nagorsen & Peterson, 1980); 'intermediate', with the wings only partly folded so that the individual digits are distinguishable (Wagstaffe & Fidler, 1968) and 'extended', with one wing fully extended and the other fully folded (Hangay & Dingley, 1985). Although fluid-preserved bats are only routinely preserved in the compressed position (Nagorsen & Peterson, 1980), it is instructive to use all of these positions for fluid-preserved specimens to test whether the position in which the wing is fixed affects wing area values as determined from preserved specimens.

Norberg & Rayner (1987) compiled a list of wing variables and selected morphological indices for all previously studied bats from personal data and/or literature sources. In so doing, they corrected for differences in procedure and definitions of the variables used. However, they did not account for the possible discrepancies caused by the use of different specimen types by the various researchers since they had no baseline information to permit them to do so. Specimen types included in Norberg & Rayner's study (1987) were standard study skins (not examined in the present study), live animals, freshly killed specimens, and alcohol-preserved specimens. Values from these various specimen types may not be comparable due to the potential problems outlined above.

Of all of the morphological characters used to predict flight performance in bats, wing area is arguably the most important as it is a necessary component of both aspect ratio and wing loading (Vaughan, 1959; Norberg & Rayner, 1987). Thus, it is important to obtain an accurate value for the wing area when carrying out such studies. The traditional and generally accepted standard for determining wing area is to trace its outline on to a sheet of paper. The area of the outline is then calculated using a polar planimeter (e.g. Poole, 1936) or a digitizing pad attached to a computer (e.g. Aldridge & Brigham, 1988).

However, these tracing procedures have recently been criticized as being too time consuming, potentially damaging to museum specimens, and potentially inaccurate for museum specimens whose wings have not been fixed in an extended position (Smith & Starrett, 1979; Blood & McFarlane, 1988). Procedures initially proposed as being simpler, albeit less accurate (Pirlot, 1977) alternatives to the tracing procedure, may not be subject to the problems affecting the latter when fluid-preserved specimens are involved. These 'estimation procedures' (e.g. Findley *et al.*, 1972; Pirlot, 1977; Smith & Starrett, 1979; Aldridge, 1988; Blood & McFarlane, 1988) seek to estimate wing area from a few easily obtainable linear measurements (e.g. forearm length, wingspan and lengths of digits III, IV and V) by assuming it to be a combination of simple geometric figures. As the wing does not need to be stretched out in these procedures, the value obtained for the wing area should be independent of the effects of formalin fixation on the collagen network of the wing and wing joints. However, discrepancies when compared to the live value of the wing area may still be present as it has been documented that even dimensions of essentially bony elements are affected by the preservation process (Lee, 1982).

The purpose of this study was two-fold: first, to quantify the effects of fixation and preservation on the assessment of various wing variables in chiropteran specimens (morphometric portion);

and secondly, to assess the accuracy of four representative estimation procedures (Pirlot, 1977; Smith & Starrett, 1979; Aldridge, 1988; Blood & McFarlane, 1988) on the different specimen types (estimation portion). To accomplish both objectives, individuals of a single species of bat, the little brown bat (*Myotis lucifugus*), were fixed and then stored in various preservatives and in various wing positions, according to standard museum techniques. Measurements were taken from living, freshly killed, and preserved specimens, the latter being measured at intervals from zero to 36 weeks post-fixation. Any changes in wing area values were expected to arise as a result of the effects of the fixative, the preservatives, and/or the fixation positions of the flight membrane itself (by deformation of the collagen network), by changes in the flexibility of the joints (thus indirectly affecting the assessment of wing area), or through potential bone and/or cartilage shrinkage (Arata, 1968). The accuracy of the estimation procedures was examined by comparing the wing area value determined by a given estimation procedure to the average wing area determined from tracings of all 26 live bats.

## Methods and materials

### *Specimen collection*

Live *M. lucifugus* were obtained by mist-netting in and around Calgary, Alberta from June to August, 1989 (7 bats) or by hand from a hibernaculum (Cadomin Caves) near Hinton, Alberta in mid-October, 1989 (19 bats). *Myotis lucifugus* was chosen for this study as it is common in Alberta and because its morphology and flight characteristics have been fairly well documented (Aldridge, 1986, 1988; Norberg & Rayner, 1987). Only adults were taken, since the metacarpal-phalangeal joints are not yet ossified in juveniles (Anthony, 1988), and thus may not behave in the same way throughout the preservation process as those of adults. Although *M. lucifugus* is sexually dimorphic (Myers, 1978), males were preferentially taken in order to minimize long-term effects on the population.

### *Preparation of specimens*

As far as possible, specimens were prepared according to standard museum techniques. The total sample of 26 bats was subdivided into 6 sub-samples according to preservation fluid and fixed wing position of the specimen (Table I).

All bats caught by mist-net were kept alive overnight (approximately 8 h) in small cotton 'bat bags' to ensure that their digestive tracts were empty, thereby eliminating the effects of dietary mass on the variables derived from body mass (wing loading and minimum power speed) (Buchler, 1975; Aldridge, 1986). This step was unnecessary for bats taken from the hibernaculum. After killing the animals by cervical dislocation, the peritoneal cavity of each specimen was injected with 10% neutral buffered formalin until turgid. The entire

TABLE I

*Sample sizes and capture location information of* ***M. lucifugus*** *specimens divided into preservation fluid and wing fixation position sub-samples. Sample sizes given as number from Calgary/number from Cadomin Caves*

| Sub-sample | | | | | |
|---|---|---|---|---|---|
| 70% Ethanol | | | 45% Isopropyl Alcohol | | |
| Compressed | Extended | Intermediate | Compressed | Extended | Intermediate |
| 5/0 | 1/4 | 1/3 | 0/4 | 0/4 | 0/4 |

specimen was subsequently fixed in 10% neutral buffered formalin for approximately 48 h (Nagorsen & Peterson, 1980). Although this 'stick-injection' method has been criticized as being inferior to intracardiac perfusion (Bhatnagar, 1988), it is the predominant method for fixing museum specimens (Nagorsen & Peterson, 1980), and such specimens have formed the primary source of material for study.

Prior to fixation, the specimen's wings were placed in either the compressed, intermediate, or extended position. Two pairs of microscope slides were clamped on either side of the left wing with bulldog clamps to facilitate full wing extension in the extended specimens. The clamps were then pinned to a dissecting tray. Although wings are typically fixed in the extended position by pinning, our technique was adopted to avoid puncturing the wing membrane. It was found that the weight of the wings themselves was sufficient to keep them in the compressed or intermediate positions during fixation. The uropatagium (tail membrane) was extended as much as possible and weighed down with a microscope slide. The thumb was directed forward. In order to maintain a formalin-saturated atmosphere during fixation, all specimens were covered with formalin-soaked paper towels and placed in a sealed container.

Subsequent to fixation, specimens were rinsed in standing water for approximately 24 h (Nagorsen & Peterson, 1980) and then transferred to the appropriate preservation fluid (70% ethanol or 45% isopropyl alcohol). Compressed and intermediate specimens were stored in standard specimen jars, while extended specimens were placed in Rubbermaid® trays.

### *Definitions of morphological measurements*

Morphological data were gathered from the specimens by measuring mass and tracing the left side of the specimens on paper to determine wing area and wingspan. The measurement of mass indicated whether any changes were induced by fluid preservation (dehydration vs. distension). Live bats were weighed using a 30-g Pesola scale ($\pm 0{\cdot}5$ g) while all other stages were weighed with an electronic scale ($\pm 0{\cdot}001$ g). Wet specimens (see below) were blotted before weighing.

As noted by Davis (1969), the term 'wing area' is often misleading as it frequently includes the area of the uropatagium, body and occasionally the head, in addition to the wing. Hereafter we will use the term 'lifting surface area' (LSA) for this entire complex, leaving wing area to describe the surface area of the wings alone. For this study, we adopted Norberg's (1981) definition of LSA: the combined area of the 2 wings, the entire uropatagium, and the body area between the wings, but excluding the head (Fig. 1a). One-half of the wingspan was measured as the length of the line running from the body midline to the wing tip and passing through the shoulders and the base of the thumb (Fig. 1a). Wingspan was taken to be twice this length.

### *Tracing and measurement protocol*

Tracings of the left wing were made from live bats, freshly killed specimens, specimens immediately following fixation and rinsing (post-fixation), and at 2-week intervals during the preservation process (up to 36 weeks).

A generalized tracing procedure was used to determine LSA and wingspan. After being sufficiently blotted to remove excess fluid (not necessary for live or freshly killed specimens), the specimens were placed in a supine position on a sheet of paper (as opposed to prone in Davis, 1969) to eliminate rolling about the rib cage and to keep the wing as flat to the paper as possible. The left wing was stretched to its fullest extent without damaging it and the left hind limb and the uropatagium were also stretched out and held flat. The position of the shoulders (gleno-humeral joint) was marked and the outline of the wing and corresponding half of the uropatagium as far as the tail tip was traced. All specimens were traced twice at each stage (Saunders, 1989).

The values of LSA and wingspan were determined ($\pm 1{\cdot}0$ mm$^2$ and $\pm 1{\cdot}0$ mm, respectively) using Sigma Scan V3.0® on a Zenith data systems PC with Houston Instrument Hipad™ digitizing pad. Both LSA and wingspan were outlined 3 times for a given tracing, with the resulting mean values being doubled to generate

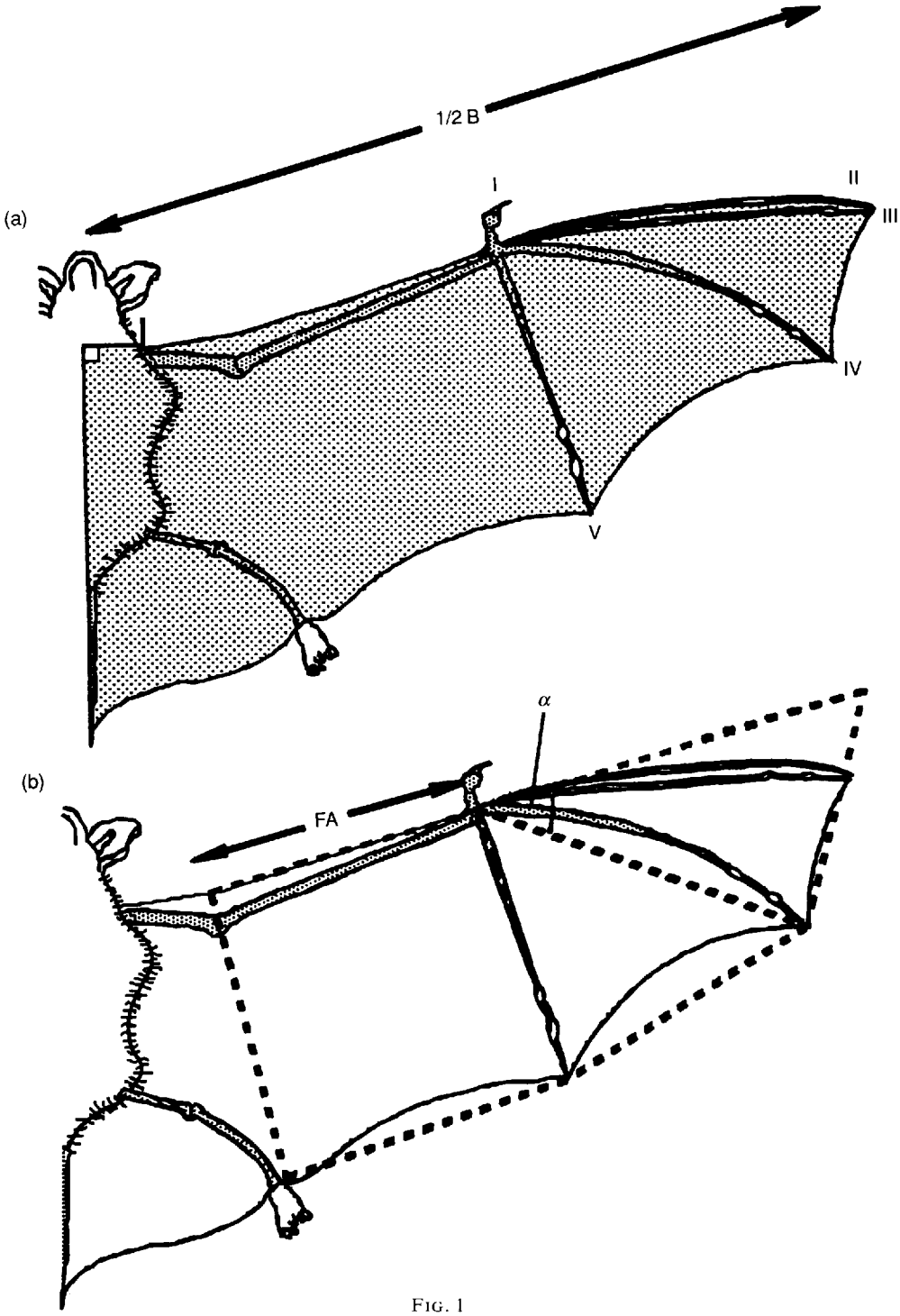


FIG. 1

the value for the whole animal. The value for a given stage for a specimen was recorded as the average of the mean values of the 2 tracings.

### *Definitions of morphological indices and flight speeds*

At each stage, 2 morphological indices (aspect ratio and wing loading) and an ecologically important flight speed (minimum power speed) were determined for each specimen using LSA (S, in $m^2$), wingspan (B, in m), and/or mass (M, in kg).

Aspect ratio (A) is calculated as the ratio of wingspan squared to the LSA for wings with an irregular outline, such as bat wings (Eq. 1) (Vaughan, 1959; Norberg & Rayner, 1987).

$$A = B^2/S \tag{1}$$

Wing loading ($Q_s$) is calculated as body mass multipled by the acceleration due to gravity, all divided by LSA (Eq. 2) (Norberg & Rayner, 1987). The body mass of the freshly killed specimen was used to calculate wing loading for all stages.

$$Q_s = (M \times 9{\cdot}81 \text{ m/sec}^2)/S \tag{2}$$

Minimum power speed ($V_{mp}$) was calculated using the equation presented by Norberg & Rayner (1987) (Eq. 3). Again, the body mass from the freshly killed specimen was used to calculate the flight speed for all stages.

$$V_{mp} = 6{\cdot}58\ M^{0{\cdot}422}B^{-0{\cdot}479}S^{-0{\cdot}148} \tag{3}$$

### *Definitions of estimation procedures*

The formulae used in this study (Eqs 4–7) are identical to those used in the original papers, except for the following changes. Pirlot's (1977) method suggested a correction factor of 0·72–0·75 for vespertilionids. We simply used the midpoint of this range, 0·735. As noted by Blood & McFarlane (1988), Smith & Starrett's (1979) formula for the handwing contained a simple geometric error. The triangles used to approximate the handwing were calculated as base multipled by height (Smith & Starrett, 1979), not one-half base times height. We here employ the corrected version of one-half base times height.

The 3 digit lengths (III, IV, V, in m) were measured from the base of the thumb to the end of the respective digit (Fig. 1a). Forearm length (FA, in m) was taken as the distance from the outside of the wrist to the outside of the elbow on the folded right wing (DeBlase & Martin, 1981) (Fig. 1b).

$$LSA = 0{\cdot}735\ BV; \text{ Pirlot (1977)} \tag{4}$$

$$LSA = 2[(FA \times V) + \tfrac{1}{2}(V \times III)]; \text{ Blood \& McFarlane (1988)} \tag{5}$$

$$LSA = 2[(FA \times V) + \tfrac{1}{2}(\cos(\alpha) \times III \times IV) + \tfrac{1}{2}(\sin(\alpha) \times IV \times V)]; \text{ modified from Smith \& Starrett (1979)}$$

where:

$$\alpha = \arctan(V/III) \text{ (see Fig. 1b); Smith \& Starrett (1979)} \tag{6}$$

$$LSA = (2{\cdot}85 \times 10^{-3})M^{2/3}; \text{ Aldridge (1988)} \tag{7}$$

These definitions of LSA differ between the authors. Both Pirlot (1977) and Aldridge (1988) define LSA as outlined in Norberg (1981): the combined area of the 2 wings, the entire uropatagium, and the body area

---

FIG. 1. Diagrammatic representations of *M. lucifugus*. (a) Boundaries of one-half of the lifting surface area (▨) and definition of one-half of the wingspan ($\frac{1}{2}$B). Digits are indicated by Roman numerals. (Adapted from Saunders (1989).) (b) Comparison of one-half of the bat wing with an idealization of the bat wing typical of many estimation procedures (– – –). FA = forearm length, $\alpha$ = Smith & Starrett's (1979) alpha angle. (Adapted from Smith & Starrett (1979) and Saunders (1989).)

between the wings, but excluding the head. Smith & Starrett (1979) define LSA as the area of the wings alone (i.e. chiropatagium, plagiopatagium and propatagium) while Blood & McFarlane (1988) define it as the area of the chiropatagium and plagiopatagium only. When comparisons were made with the live traced LSA, each author's estimate of LSA was compared to an analogous traced LSA, except for that of Blood & McFarlane (1988) which was compared to wing area (i.e. including the propatagium) since the area of the propatagium could not be extracted from our tracings of the live bats.

### *pH of alcohols*

Recalling Viidik's (1980) finding that pH affects the collagen network of some collagenous tissues, the pHs of the alcohols used in this study were measured. Both stock solutions and the alcohol in which the specimens were bathing after 42 weeks were analysed using an Orion 960 Autochemistry system. The specimen solutions were again measured at 86 weeks using a Radiometer Copenhagen PHM 71 Mk2 Acid-Base Analyzer. For specimen solutions, samples were taken from all specimen containers (3 for 70% ethanol and 4 for 45% isopropyl alcohol). Each sample was measured 3 times and the results averaged for a given solution type.

### *Statistical analysis*

The initial hypothesis for the morphometric portion of this study was that there would be no differences in any of the variables (LSA, wingspan, mass, aspect ratio, wing loading and minimum power speed) between the various stages in the prevention process, the fixation position of the wing, the preservation fluids used, and/or the capture location of the bat. The initial hypothesis for the estimation portion of this study was that there would be no difference in the LSA of live bats as determined by tracing procedures and the estimated analogous LSAs of a given specimen type as determined by Pirlot's (1977), Blood & McFarlane's (1988), Smith & Starrett's (1979) and Aldridge's (1988) estimation procedures. All statistical tests were carried out using Systat 5.0 (Wilkinson, 1990) on an IBM PC. A rejection level of 0·05 was used. For statistical tests involving multiple comparisons the rejection level of 0·05 was corrected using the Bonferroni method (Eq. 8) (Snedecor & Cochran, 1989):

$$\alpha_C = \alpha_E / r \tag{8}$$

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

A 3-factor (preservation fluid, wing position, capture location) repeated measures analysis of variance (ANOVA) was performed on the dataset over the stages of preservation in order to discriminate the possible effects of preservation fluid, wing position, capture location, and/or individual variation on any of the 6 variables examined. Of the alcohol-preserved specimens, only the 36-week stage was examined in the ANOVA. Aldridge's (1988) method was not analysed with an ANOVA since it relies on mass alone to estimate LSA (Eq. 7) and thus would behave similarly. For all 4 estimation procedures, the information from the ANOVAs was used only to determine how the bats would be grouped (e.g. by wing position) when being compared to the live traced LSAs.

Tukey honestly significant difference (HSD) tests were used to detect differences between specimens of the same stage but of different wing, alcohol, or capture location groupings. Two-tailed, 2 sample Student's *t*-tests were used to compare: (1) values from live and 36-week specimens of a given wing or alcohol grouping; (2) traced LSA of live bats and the estimated analogous LSAs of a given specimen type; and (3) alcohol pHs (Zar, 1984).

## Results

### *Effects on morphometry*

The two samples of *Myotis lucifugus* used in this study (Calgary vs. Cadomin Caves) differed significantly from each other. All variables showed a capture location effect in the ANOVA

TABLE II

*F-ratio for Wilks' lambda statistic of three-factor repeated measures analysis of variance over preservation stage testing hypothesis of no effect of a given factor(s) on various variables of* ***M. lucifugus*** *specimens. Factors are stage—specimen type; alcohol—preservation fluid; wing—wing fixation position of specimen; and location—capture location of specimen. (**n** = 26 for each variable, ***P** < 0·05, ****P** < 0·01, *****P** < 0·001, d.f. = degrees of freedom)*

| | Factor | | | | |
|---|---|---|---|---|---|
| Variable | Stage (*d.f.* = 3) | Stage*Location (*d.f.* = 3) | Stage*Alcohol (*d.f.* = 3) | Stage*Wing (*d.f.* = 6) | Stage*Alcohol*Wing (*d.f.* = 6) |
| LSA | 115·12*** | 6·54** | 2·37 | 5·32** | 0·39 |
| B | 209·28*** | 8·47** | 1·43 | 8·77*** | 0·87 |
| M | 233·19*** | 2·30 | 6·93** | 1·89 | 2·58* |
| A | 20·00*** | 6·54** | 0·17 | 2·67* | 0·61 |
| $Q_s$ | 75·14*** | 7·06** | 1·34 | 3·53** | 0·36 |
| $V_{mp}$ | 209·99*** | 6·59** | 1·86 | 7·62*** | 1·16 |
| Pirlot (1977) | 122·10*** | 5·46** | 0·82 | 5·16** | 0·68 |
| Blood & McFarlane (1988) | 77·78*** | 6·08** | 0·08 | 5·12** | 0·76 |
| Smith & Starrett (1979) | 116·99*** | 6·38** | 0·42 | 4·93** | 0·98 |

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

analysis, either singly (i.e. location; F = 8·02 (LSA), 14·76 (B) and 9·96 (M); *d.f.* = 1; $P < 0·05$ (LSA) and $P < 0·01$ (B and M)) and/or in combination with a stage effect (i.e. Stage*Location) (Table II). Only LSA (live), aspect ratio (freshly killed and 36 weeks), and wing loading (36 weeks) revealed no significant differences between the populations with respect to capture location (Table III).

With the exception of mass, all variables (Fig. 2 (a, b), and (d–f)) displayed a wing effect (i.e. the value of the variable was dependent on the fixation position of the wing). In all cases, this was due to a combination Stage*Wing effect (Table II) with wingspan showing a lone wing effect as well (F = 5·16, *d.f.* = 2, $P < 0·05$). Significant differences existed between the compressed and extended bats for all variables (except mass) at the post-fixation stage (Table III). In addition, significant differences existed between the extended and intermediate bats for aspect ratio (36 weeks) and $V_{mp}$ (36 weeks) and between the compressed and intermediate bats for aspect ratio (36 weeks) (Table III). Significant differences between the live and 36-week stages only existed for the

TABLE III

*T statistics from Tukey honestly significant difference tests testing null hypothesis of no difference between values of a given variable at a given stage when grouped by: (a) capture location; (b) wing fixation position; and (c) preservation fluid. (*P < 0·05, **P < 0·01, ***P < 0·001)*

(a) *By capture location—Calgary vs. Cadomin Caves:*

| | Stage | | | |
|---|---|---|---|---|
| | Live | Freshly killed | Post-fixation | 36 Weeks |
| LSA | −0·31 | −5·41*** | −7·73*** | −6·77*** |
| B | −2·34* | −3·65** | −8·55*** | −5·70*** |
| M | 7·65*** | 6·41*** | 8·28*** | 9·20*** |
| A | −2·98** | −1·66 | −6·77*** | −0·69 |
| $Q_s$ | 5·72*** | 3·84** | 2·18* | 1·54 |
| $V_{mp}$ | 5·58*** | 4·70*** | 3·75** | 2·11* |

TABLE III (*cont.*)

(bi) *By wing fixation position—compressed vs. extended:*

| | Stage | | | |
|---|---|---|---|---|
| | Live | Freshly killed | Post-fixation | 36 Weeks |
| LSA | 1·92 | 2·51 | 22·75** | 10·95* |
| B | 0·18 | 0·32 | 4·20** | 1·28 |
| A | 0·17 | 0·02 | 0·69* | 0·04 |
| $Q_s$ | 0·77 | 0·53 | 1·24** | 0·03 |
| $V_{mp}$ | 0·14 | 0·11 | 0·29** | 0·01 |

(bii) *By wing fixation position—compressed vs. intermediate:*

| | Stage | | | |
|---|---|---|---|---|
| | Live | Freshly killed | Post-fixation | 36 Weeks |
| LSA | 2·55 | 1·27 | 10·29 | 5·01 |
| B | 0·11 | 0·02 | 1·89 | 0·05 |
| A | 0·06 | 0·06 | 0·30 | 0·32** |
| $Q_s$ | 0·62 | 0·43 | 0·35 | 0·22 |
| $V_{mp}$ | 0·12 | 0·10 | 0·10 | 0·09 |

(biii) *By wing fixation position—extended vs. intermediate:*

| | Stage | | | |
|---|---|---|---|---|
| | Live | Freshly killed | Post-fixation | 36 Weeks |
| LSA | 0·63 | 1·25 | 12·46 | 5·94 |
| B | 0·29 | 0·30 | 2·31 | 1·32 |
| A | 0·10 | 0·08 | 0·39 | 0·29** |
| $Q_s$ | 0·16 | 0·10 | 0·90 | 0·25 |
| $V_{mp}$ | 0·02 | 0·01 | 0·20* | 0·08 |

(c) *By preservation fluid—70% ethanol vs. 45% isopropyl alcohol:*

| | Stage | | | |
|---|---|---|---|---|
| | Live | Freshly killed | Post-fixation | 36 Weeks |
| M | 4·53*** | 2·99** | 3·90** | 4·36*** |

For definition of variables see Table II

compressed bats for both LSA ($\alpha_C = 0{\cdot}003$, $t = 4{\cdot}23$, $d.f. = 16$, $P = 0{\cdot}001$) and aspect ratio ($\alpha_C = 0{\cdot}003$, $t = -3{\cdot}60$, $d.f. = 16$, $P = 0{\cdot}002$). Bats in the extended position showed the least amount of variation between the different stages, with the compressed and intermediate bats showing noticeably greater amounts of variation (Fig. 2 (a, b), and (d f)).

Only mass (Fig. 2c) was subject to an alcohol effect, both alone ($F = 7{\cdot}51$, $d.f. = 1$, $P = 0{\cdot}05$) and in concert with a stage effect (Table II). The ethanol and isopropyl bats were significantly different from each other for all stages (Table III). The isopropyl bats showed a significant increase from the live to the 36-week stage ($\alpha_C = 0{\cdot}003$, $t = -7{\cdot}05$, $d.f. = 22$, $P = 0{\cdot}000$). A combination Stage*Alcohol*Wing effect was also present for mass (Table II).

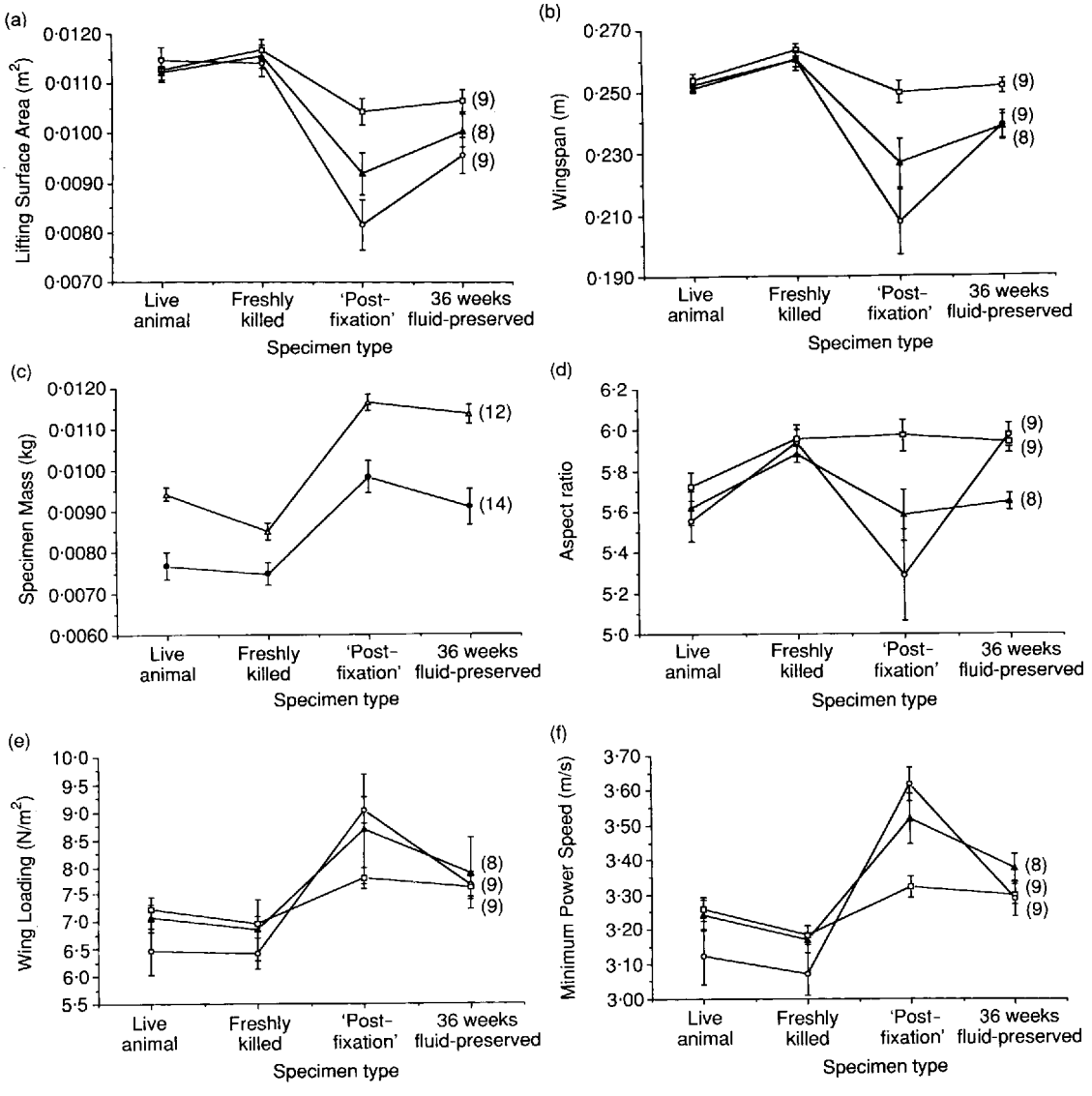


FIG. 2. Changes in morphometric variables during the preservation process: (a) lifting surface area; (b) wingspan; (c) specimen mass; (d) aspect ratio; (e) wing loading; and (f) minimum power speed. For all variables except mass, bats were grouped according to wing position (○--○, compressed; ▲—▲, intermediate; □—□, extended); for mass, bats were grouped according to preservation fluid (●—●, 70% ethanol; △—△, 45% isopropyl alcohol). Groups presented as means ±S.E. Numbers in parentheses refer to sample sizes.

### *Effects on estimation procedures*

Except for the Aldridge (1988) procedure, all of the variables examined in this study were subject to a location effect (i.e. the value of the variable was dependent upon the capture location of the bat). This location effect was manifested as a Stage*Location effect (Pirlot, 1977; Blood &

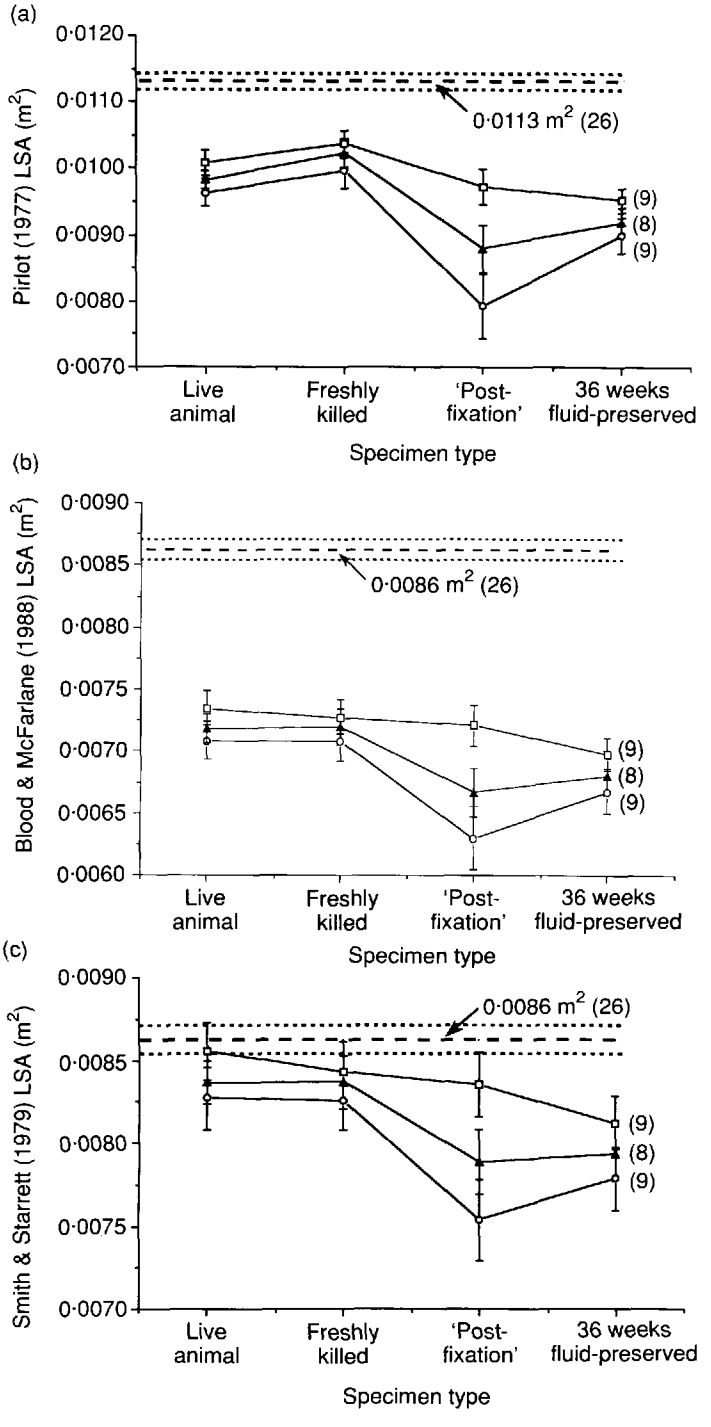


FIG. 3. Changes in lifting surface area obtained from various estimation procedures: (a) Pirlot (1977); (b) Blood & McFarlane (1988); and (c) Smith & Starrett (1979). Bats were grouped according to wing position (○—○, compressed; ▲—▲, intermediate; □—□, extended). Groups presented as means ± S.E. Live traced lifting surface area presented as (– – –) ± S.E. Numbers in parentheses refer to sample sizes.

McFarlane, 1988; Smith & Starrett, 1979; Table II) and/or as a lone location effect (Pirlot, 1977; $F = 5{\cdot}77$, $d.f. = 1$, $P < 0{\cdot}05$).

In addition, all of the variables except that of Aldridge (1988) displayed significant Stage*Wing effects (i.e. the value of the variable was dependent upon both the fixed wing position and the type of specimen that was measured) (Table II). Thus, the methods of Pirlot (1977), Blood & McFarlane (1988), and Smith & Starrett (1979) had their estimated values grouped by wing position for the 36-week preserved stage for comparisons with the live traced LSA.

The mean value ($\pm$S.E.) for the live wing area was $0{\cdot}0086 \pm 8{\cdot}50 \times 10^{-4}$ m$^2$ ($n = 26$), while for the Norberg (1981) definition of LSA it was $0{\cdot}0113 \pm 1{\cdot}26 \times 10^{-4}$ m$^2$ ($n = 26$). The methods of both Pirlot (1977) and Blood & McFarlane (1988) always yielded results significantly different from their analogous live traced LSAs ($\alpha_C$ for this section $= 0{\cdot}004$, $t = -8{\cdot}85$, $d.f. = 50$, $P = 0{\cdot}000$ and $t = -11{\cdot}99$, $d.f. = 50$, $P = 0{\cdot}000$, respectively). In every case, the Pirlot (1977) and Blood & McFarlane (1988) methods on 36-week preserved specimens underestimated their analogous live LSAs (Fig. 3a, b); compressed: $t = -8{\cdot}78$, $d.f. = 33$, $P = 0{\cdot}000$ and $t = -11{\cdot}05$, $d.f. = 33$, $P = 0{\cdot}000$, respectively; extended: $t = -7{\cdot}62$, $d.f. = 33$, $P = 0{\cdot}000$ and $t = -10{\cdot}13$, $d.f. = 33$, $P = 0{\cdot}000$, respectively; and intermediate: $t = -8{\cdot}29$, $d.f. = 32$, $P = 0{\cdot}000$ and $t = -10{\cdot}34$, $d.f. = 32$, $P = 0{\cdot}000$, respectively). Significant differences from the analogous LSA for Smith & Starrett's (1979) method were restricted to compressed and intermediate bats at the 36-week stage ($t = -4{\cdot}61$, $d.f. = 33$, $P = 0{\cdot}000$ and $t = -3{\cdot}88$, $d.f. = 32$, $P = 0{\cdot}000$, respectively), which again were underestimates of the true LSA (Fig. 3c). Values obtained from Aldridge's (1988) estimation procedure (mean $\pm$ S.E. $= 0{\cdot}0118 \pm 2{\cdot}45 \times 10^{-4}$ m$^2$, $n = 26$) were not significantly different with respect to the analogous live traced LSA ($t = 1{\cdot}78$, $d.f. = 37{\cdot}3$, $P = 0{\cdot}081$).

### *pH of alcohols*

The pHs of stock 70% ethanol and 45% isopropyl alcohol (Table IV) differed significantly ($\alpha_C$ for this section $= 0{\cdot}006$, $t = 46{\cdot}61$, $d.f. = 2{\cdot}1$, $P = 0{\cdot}000$). Such was also the case with the 70% ethanol and 45% isopropyl alcohol in which the specimens had been bathing for either 42 ($t = 18{\cdot}37$, $d.f. = 19$, $P = 0{\cdot}000$) or 86 weeks ($t = 14{\cdot}08$, $d.f. = 19$, $P = 0{\cdot}000$). Except for the stock and 86-week 70% ethanol, the specimen alcohols differed significantly from each other (ethanol, 42 vs. 86 weeks: $t = 9{\cdot}56$, $d.f. = 11{\cdot}3$, $P = 0{\cdot}000$; isopropyl alcohol, 42 vs. 86 weeks: $t = 9{\cdot}51$, $d.f. = 22$, $P = 0{\cdot}000$) and their respective stock solutions (ethanol, 42 weeks vs. stock: $t = 22{\cdot}21$, $d.f. = 8{\cdot}9$, $P = 0{\cdot}000$; isopropyl alcohol, 42 weeks vs. stock: $t = 26{\cdot}85$, $d.f. = 13$, $P = 0{\cdot}000$; 86 weeks vs. stock: $t = 29{\cdot}37$, $d.f. = 13$, $P = 0{\cdot}000$).

TABLE IV

*pHs of stock 70% ethanol and 45% isopropyl alcohol, and the alcohol surrounding the specimens after 42 weeks and 86 weeks*

| Solution | *n* | Mean pH | S.E. |
|---|---|---|---|
| 70% stock ethanol | 3 | 5·90 | $3{\cdot}33 \times 10^{-3}$ |
| Ethanol at 42 weeks | 9 | 6·20 | $1{\cdot}35 \times 10^{-2}$ |
| Ethanol at 86 weeks | 9 | 5·90 | $2{\cdot}89 \times 10^{-2}$ |
| 45% stock isopropyl alcohol | 3 | 4·53 | $2{\cdot}91 \times 10^{-2}$ |
| Isopropyl alcohol at 42 weeks | 12 | 4·53 | $2{\cdot}05 \times 10^{-2}$ |
| Isopropyl alcohol at 86 weeks | 12 | 5·48 | $1{\cdot}44 \times 10^{-2}$ |

## Discussion

Fluid-preserved museum specimens provide invaluable data for research on bats, but some care must be exercised when interpreting data obtained from such specimens. The collection locality of our bats confounded the preservation effects we observed. The two samples of bats used in this study (Calgary and Cadomin Caves) consistently differed from one another throughout the preservation process for all of the variables examined. The difference is likely due to dissimilarities between the samples themselves, since potential variation within the preservation process was minimized by having all specimens collected within two months of one another, and prepared and traced by the senior author. The problem this sample heterogeneity creates for data compilations such as those of Norberg & Rayner (1987) are obvious, even without inclusion of different preservation techniques. Such compiled datasets are probably not homogeneous, and even datasets from individual researchers may not be homogeneous due to this phenomenon.

The choice of alcohol is not an important consideration with respect to accurate determination of any of the variables examined. Only mass was subject to an alcohol effect, but this finding is dubious. The observed alcohol effect is more likely a size effect, as significant differences between the respective alcohol specimens were found before the specimens were even immersed in alcohol (e.g. the live, freshly killed and post-fixation stages). An analysis of covariance (ANCOVA) designed to minimize size effects yielded less satisfactory results, such as revealing wing effects on mass that could not be explained. In this study, the Stage*Alcohol*Wing effect observed for mass appears to be a carryover of the highly significant Stage*Alcohol effect (Table II).

The apparent lack of any alcohol effects could be due to the short-term nature of this study. Both alcohols are weakly acidic and the acidic degradation of the collagen network noted by Viidik (1980) might only occur over the very long term. Autoxidation of the alcohols will make them increasingly acidic with time (Korcek *et al.*, 1972), enhancing the degradative process. The initial increase in pH at 42 weeks was probably due to the leaching of the fairly neutral body fluids and water (from the post-fixation rinsing) into the alcohol, diluting it before autoxidation began to bring the pH down.

Although we cannot assess the long-term effects of the slightly acidic alcohols, our results show that other changes do occur over the short term. The widespread stage effects indicate that a variable's value is dependent on the specimen type from which it was obtained, even over a period of only nine months. A further complication is manifested by differences within a specimen type according to the fixation position of the wings. As would be predicted, significant differences between the wing types occurred only in the preserved specimens, after the wings had been fixed. In most cases, the differences were between the extreme wing positions, compressed and extended. In fact, despite being the standard wing fixation position, the compressed position was arguably the worst of the three as it was the only one to generate significant differences between the live and 36-week stages.

The exact nature of the changes induced by the fixation procedure were beyond the scope of this study. The wing effects could result from the deformation of the collagen network by formalin (Viidik, 1980) and/or increased joint stiffness. Both factors would make the compressed and intermediate wings more difficult to stretch out fully. Further study in this area is required. The structure of the collagen network of the wing during fixation and preservation could be followed using electron microscopy (e.g. Holbrook & Odland, 1978) or biomechanical testing techniques (e.g. Viidik, 1980). The amount of resistance in the joints could also be measured.

Given that differences do occur between specimens of various types and wing fixation positions,

it is also possible that such differences may affect the predictions of flight performance for bats made from museum specimens. We have shown that aspect ratio, wing loading and $V_{mp}$ are all subject to stage effects and that aspect ratio values from 36-week compressed bats differ significantly from their live values.

As was the case for many primary morphological variables (LSA, wingspan and mass), the capture location of the bat played an important role in the determination of estimated LSA. The inherent inaccuracies of the estimation procedures (see below) were not large enough to obscure the differences in LSA that apparently exist between different populations of bats. While this does not mean that a bat from a given location will yield inaccurate estimates of LSA (with respect to the live traced LSA value), it does present similar problems to those associated with the morphologic variables.

Estimation procedures offer a trade-off between speed and accuracy when compared to tracing procedures. As most estimation procedures employ idealized representations of the bat wing, they will over- and under-represent different sections of the wing (see Fig. 1b). As this cannot be avoided, the different procedures seek to minimize these misrepresentations by applying an appropriate correction factor (e.g. Pirlot, 1977) or by increasing the number of estimated sections to represent the different sections of the wing more accurately (e.g. Smith & Starrett (1979) vs. Blood & McFarlane (1988) vs. Pirlot (1977)).

Of the three estimation procedures based on an idealization of the bat wing, both Pirlot's (1977) and Blood & McFarlane's (1988) were clearly unacceptable for estimating the LSA of *M. lucifugus*. Both procedures significantly underestimated the value of the LSA they were attempting to estimate (Norberg's (1981) definition and wing area, respectively) in all specimen types. Pirlot's method can be easily corrected by increasing the correction factor; however, this is undesirable as a suitable correction factor for every bat species may need to be found.

The underestimates yielded by Blood & McFarlane's method could be due to the fact that their estimated values of LSA were not compared to their definition of LSA. However, they are the only authors we know of that exclude the propatagium from the flight surface, so their LSA values (wing area minus propatagial area) cannot be easily compared with values from other authors. Blood & McFarlane approximate the armwing as a simple rectangle. Yet the plagiopatagium alone is not as good a rectangle as is the plagiopatagium plus the propatagium. In any case, Blood & McFarlane's formula for the armwing is identical to that of Smith & Starrett (1979), which includes the propatagium. Thus, the greater underestimates yielded by Blood & McFarlane's method (as compared to Smith & Starrett's) must arise from their idealization of the handwing as a single right triangle.

Only Aldridge's (1988) and Smith & Starrett's (1979) procedures accurately estimated their respective LSAs some of the time for *M. lucifugus*. Aldridge's formula is derived using data from birds, not bats (Aldridge, 1988), but this does not seem to detract from its accuracy. However, its accuracy is limited to masses obtained from live animals, and not preserved specimens. We did not test Aldridge's formula using masses obtained from preserved specimens as these masses are never measured; it is accepted that these masses are different from the live mass. Thus, the use of Aldridge's (1988) formula is limited with regard to museum specimens to those where the live (or freshly killed) mass is known.

Smith & Starrett's (1979) estimation procedure was the only one of the wing-idealization procedures to yield an accurate estimate of the LSA of live bats and also worked for 36-week preserved bats whose wings were fixed in the extended position. This result is yet another argument in favour of using the extended wing position for fluid-preserved bat specimens, as opposed to the

more common compressed position. Only fluid-preserved bats fixed in the extended position will allow accurate determination of LSA by either tracing procedures or Smith & Starrett's estimation procedure. In addition, the use of Smith & Starrett's procedure (as well as Aldridge's (1988)) might prove advantageous for field workers. Although it is possible to perform tracings of bat wings in the field (e.g. Saunders, 1989), Smith & Starrett's procedure adds only three easily obtained measurements (lengths of digits III, IV and V) to the list of standard measurements that are already taken for bats.

We should add a cautionary note that these results are really only justified for *M. lucifugus*. By using mass (volume) to estimate LSA (area), Aldridge's (1988) formula may be subject to allometric effects when used on different-sized bat species: smaller bats have proportionally larger wings relative to their body size (indicated by mass) than do larger bats (Lawlor, 1973). However, these scaling differences may not be serious as most birds are larger than *M. lucifugus*, and Aldridge's formula appears to work equally well on both.

The use of two sections for the chiropatagium (as opposed to only one in Blood & McFarlane's (1988) method) may make Smith & Starrett's (1979) estimation procedure adjustable to other bat species with very differently configured handwings. The only limitation with Smith & Starrett's procedure is that it estimates the area of the wing alone, whereas the standard definition of LSA today appears to be that of Norberg (1981). However, this limitation can be overcome using the correction factors for the body and uropatagium presented by Norberg & Rayner (1987).

## Summary

In order to minimize the effects of formalin fixation on bat specimens, we offer a number of suggestions. Although our results suggest the choice of alcohol is not important with respect to determination of any of the variables over the short term, it would be worthwhile to use 70% ethanol. Bats preserved in ethanol did not significantly increase in mass over the preservation process. Ethanol's less acidic nature would also minimize long-term acidic degradation of the collagen network, as well as give a longer time frame before harmfully acidic pH values are reached. Buffering the ethanol would be advantageous, but not a cure-all as Hughes & Cosgrove (1990) have found that neutral buffered formalin is not immune to pH changes.

In terms of the fixed wing position, although the intermediate and extended positions have only been suggested for study skins, they are demonstrably better than the compressed position for fluid-preserved specimens when values are compared to the live values. The extended position yields more stable results (i.e. less variation between specimen types), but is less practical. Intermediate specimens are not appreciably larger than compressed ones and do not require any extra work to fix their wings (while the extended specimens do). In light of this, we suggest that the intermediate position be used when it is not practical to use the extended position.

The estimates of LSA generated by the various estimation procedures did not generally escape the effects of fixation and preservation. Neither Pirlot's (1977) nor Blood & McFarlane's (1988) procedures are accurate for any specimen type. Use of Smith & Starrett's (1979) procedure should be limited to live or freshly-killed specimens, and those alcoholic specimens where the wings have been fixed in the extended position (another reason for museums to adopt the extended position in their collections, at least for a few individuals of each bat species). Another acceptable procedure is that of Aldridge (1988), but only for those specimens where the live or freshly killed mass is known.

We thank Mr Matt Saunders and Drs Robert Barclay, Elizabeth Dixon and Lawrence Harder for invaluable advice during the formulation of the ideas expressed herein, Mr David Bininda for allowing us access to the two pH meters, and Mr Larry Powell for providing valuable comments on the 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.

## REFERENCES

Aldridge, H. D. J. N. (1986). Manoeuvrability and ecological segregation in the little brown (*Myotis lucifugus*) and yuma (*M. yumanensis*) bats (Chiroptera: Vespertilionidae). *Can. J. Zool.* **64:** 1878–1882.

Aldridge, H. D. J. N. (1988). Flight kinematics and energetics in the little brown bat, *Myotis lucifugus* (Chiroptera: Vespertilionidae), with reference to the influence of ground effect. *J. Zool., Lond.* **216:** 507–517.

Aldridge, H. D. J. N. & Brigham, R. M. (1988). Load carrying and manoeuvrability in an insectivorous bat: a test of the 5% 'rule' of radio-telemetry. *J. Mammal.* **69:** 379–382.

Anthony, E. L. P. (1988). Age determination in bats. In *Ecological and behavioural methods for the study of bats*: 47–58. Kunz, T. H. (Ed.). Washington, D.C.: Smithsonian Institution Press.

Arata, A. A. (1968). Discrepancy between 'dry' and 'fresh' forearm measurements in bats. *J. Mammal.* **49:** 155 156.

Bhatnagar, K. P. (1988). Fixation-preservation of bats in the field. *Macroderma* **4:** 1 10.

Blood, B. R. & McFarlane, D. A. (1988). A new method for calculating the wing area of bats. *Mammalia* **52:** 600–603.

Buchler, E. R. (1975). Food transit time in *Myotis lucifugus* (Chiroptera: Vespertilionidae). *J. Mammal.* **56:** 252–255.

Davis, R. (1969). Wing loading in pallid bats. *J. Mammal.* **50:** 140–144.

DeBlase, A. F. & Martin, R. E. (1981). *A manual of mammalogy with keys to families of the world.* (2nd edn). Dubuque, Iowa: Wm. C. Brown Co. Publishers.

Farney, J. & Fleharty, E. (1969). Aspect ratio, loading, wing span and membrane area of bats. *J. Mammal.* **50:** 362 367.

Findley, J. S., Studier, E. H. & Wilson, D. E. (1972). Morphological properties of bat wings. *J. Mammal.* **53:** 429–444.

Hangay, G. & Dingley, M. (1985). *Biological museum methods.* Sydney: Academic Press.

Holbrook, K. A. & Odland, G. F. (1978). A collagen and elastic network in the wing of the bat. *J. Anat.* **126:** 21–36.

Hughes, G. W. & Cosgrove, J. A. (1990). pH changes in a formalin borax solution with inferences about uses of neutralized formalin in vertebrate collections. *Colln Forum* **6:** 21–26.

Jones, B. C. & Geen, G. H. (1977). Morphometric changes in an elasmobranch (*Squalus acanthias*) after preservation. *Can. J. Zool.* **55:** 1060–1062.

Korcek, S., Chenier, J. H. B., Howard, J. A. & Ingold, K. U. (1972). Absolute rate constants for hydrocarbon autoxidation: XXI. Activation energies for propagation and the correlation of propagation rate constants with carbon-hydrogen bond strengths. *Can. J. Chem.* **50:** 2285–2297.

Lawlor, T. E. (1973). Aerodynamic characteristics of some neotropical bats. *J. Mammal.* **54:** 71–78.

Lee, J. C. (1982). Accuracy and precision in anuran morphometrics: artifacts of preservation. *Syst. Zool.* **31:** 266–281.

Myers, P. (1978). Sexual dimorphism in size of vespertilionid bats. *Am. Nat.* **112:** 701–712.

Nagorsen, D. W. & Peterson, R. L. (1980). *Mammal collectors' manual: a guide for collecting, documenting and preparing mammal specimens for scientific research.* Toronto: Life Sciences Misc. Publ. Royal Ontario Museum.

Norberg, U. M. (1981). Allometry of bat wings and legs and comparison with bird wings. *Phil. Trans. R. Soc. (B)* **292:** 359–398.

Norberg, U. M. & Rayner, J. M. V. (1987). Ecological morphology and flight in bats (Mammalia; Chiroptera): wing adaptations, flight performance, foraging strategy and echolocation. *Phil. Trans. R. Soc. (B)* **316:** 335–427.

Pirlot, P. (1977). Wing design and the origin of bats. In *Major patterns in vertebrate evolution*: 375 410. Hecht, M. K., Goody, P. C. & Hecht, B. M. (Eds). New York: Plenum Press.

Poole, E. L. (1936). Relative wing ratios of bats and birds. *J. Mammal.* **17:** 412–413.

Rosevear, D. R. (1965). *The bats of West Africa.* London: Trustees of the British Museum (Natural History).

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.

Smith, J. D. & Starrett, A. (1979). Morphometric analysis of chiropteran wings. *Spec. Publs Mus. Texas tech. Univ.* No. 16: 229 316.

Snedecor, G. W. & Cochran, W. G. (1989). *Statistical methods.* (8th edn). Ames, Iowa: Iowa State University Press.

Struhsaker, T. T. (1961). Morphological factors regulating flight in bats. *J. Mammal.* **42:** 152–159.

Vaughan, T. A. (1959). Functional morphology of three bats: *Eumops, Myotis, Macrotus*. *Univ. Kans. Publs Mus. nat. Hist.* **12:** 1–153.

Vaughan, T. A. (1966). Morphology and flight characteristics of molossid bats. *J. Mammal.* **47:** 249–260.

Viidik, A. (1980). Interdependence between structure and function in collagenous tissues. In *Biology of collagen*: 257–280. Viidik, A. & Vuust, J. (Eds). New York: Academic Press.

Wagstaffe, R. & Fidler, J. H. (1968). *The preservation of natural history specimens* **2.** London: H. F. & G. Witherby Ltd.

Wilkinson, L. (1990). *Systat: the system for statistics*. Evanston, Illinois: Systat, Inc.

Zar, J. H. (1984). *Biostatistical analysis*. (2nd edn). Englewood Cliffs, New Jersey: Prentice-Hall, Inc.