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A novel method of estimating pterosaur skeletal mass using computed tomography scans

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Body mass is an important, basic parameter of life. It is central to understanding many aspects of an animal's ecology and behavior and is potentially the most important factor affecting locomotion performance (Biewener, 1989). Body mass is particularly significant for volant animals as it affects the speed required to take off and land, flight speed, manoeuvrability in flight and influences the upper size limit of flight (Alexander, 1998). Accordingly, accurate mass estimates are key to understanding the behavior and capability of extinct animals.

Estimates of body mass have been obtained for a number of extinct volant animals including birds (e.g., Hone et al., 2008) and pterosaurs (e.g., Bramwell and Whitfield, 1974; Brower and Veinus, 1981; Witton, 2008; Henderson, 2010). As pterosaurs were both the first vertebrates to achieve powered flight and the largest animals ever to do so, accurate mass estimates are crucial to our understanding of pterosaurian biomechanics and the upper size limits on their flight. The two main approaches that have previously been taken to calculate pterosaur body mass rely either on estimates of total body volume and density distributions (Bramwell and Whitfield, 1974; Brower and Veinus, 1981; Henderson, 2010), or the relationship between skeletal mass and total mass (Witton, 2008), using data from modern birds (Prange et al., 1979).

The results vary greatly from method to method—for example, the mass of *Quetzalcoatlus northropi* has been estimated at 75 kg (Brower and Veinus, 1981), 259 kg (Witton, 2008) and 544 kg (Henderson, 2010), although this larger estimate is thought to be based on an anatomically incorrect reconstruction (Witton and Habib, 2010). This range is so wide that it spans from an animal with an improbably low implied average body density of 0.15 g/cm³ (Witton, 2008), to a heavy animal, incapable of flight (Henderson, 2010). Even Witton's (2008) estimate of 259 kg would render the animal flightless, according to some analyses (e.g., Chatterjee and Templin, 2004), but not others (e.g., Witton and Habib, 2010).

Witton (2008) applied the skeletal mass approach to pterosaurs, estimating skeletal mass from bone volume calculated using simple geometric shapes—a

pragmatic method, and the only feasible approach in the absence of comprehensive 3D morphological information. To estimate cortical thickness, Witton (2008) applied a regression analysis of thickness-to-diameter data for a range of pterosaur bones. Then, to estimate total body mass, the relationship found by Prange et al. (1979) between skeletal mass and total mass in birds was applied, allowing determination of the body mass of various pterosaur species over a wide range of animal sizes. This relationship was deemed to be appropriate since it applies to both birds and terrestrial mammals, which are distant in phylogenetic terms, and it was therefore thought likely to be accurate for pterosaurs as well.

Here, we present a novel method that uses computed tomography (CT) scans to estimate the volume, and hence the mass of pterosaur wing bones and thus provides a more robust foundation for the methodology adopted by Witton (2008). The use of CT scans to analyze fossils is a relatively new process, but representatives of a number of different groups, including arthropods (e.g., Penney et al., 2007), mammals (e.g., Ni et al., 2012), and dinosaurs (e.g., Martinez et al., 2011), have already been studied. However, few CT studies have been performed on pterosaurs, focusing mainly on the skull (e.g., Witmer et al., 2003) or axial skeleton (Claessens et al., 2009). Only one study has reported CT scans for any part of the pterosaurian appendicular skeleton—a single humerus of *Bennettazhia* (Habib, 2008). To demonstrate the method, we estimate the masses of three first wing phalanges from different ornithocheirid pterosaur individuals, using CT scans that are new to the scientific literature.

Institutional Abbreviations—**NHMUK**, Natural History Museum of the United Kingdom, London, U.K.; **UP**, University of Portsmouth, Portsmouth, U.K.

MATERIALS AND METHODS

New CT scans from three ornithocheirid first wing phalanges (WP1; Table 1) were analyzed using the open-source software ImageJ (Abramhoff et al., 2004). As single wing phalanges are not diagnostic at the species or genus level, one specimen is tentatively identified as *Ornithocheirus* sp., while the other two can only be confidently attributed to the family Ornithocheiridae. Ornithocheirid pterosaurs were primarily marine, proportionally long-winged animals that likely used their long wings for soaring (Witton, 2013). Cortical area was calculated using the ‘Analyze Particles’ function of ImageJ (detailed CT scan information can be found in Supplementary Materials). In the shaft of the bone, the few trabeculae present were found to have negligible impact on the mass, so were ignored. In these regions, once the matrix was digitally removed (Fig. 1A), the image was binarized and adjusted to ensure only bone was included in the area calculation (Fig. 1B). The most proximal 10% and distal 20% (UP WP1 only) of the phalanges had noticeable trabeculae (Fig. 1C); these portions were analyzed more frequently to quantify the changes in cortical area between scans (Fig. 1D). In these areas where trabeculae were extensive, images were binarized manually using ImageJ’s ‘Threshold’ function to include the trabeculae while excluding matrix.

While all bones analyzed were generally well preserved, some regions had incomplete cortical bone. In these regions, the cortex was reconstructed in ImageJ using the adjacent intact sections and residual matrix as guides. Individual CT slices with significant sections missing were excluded from the analysis, to avoid introducing errors. Using the complete bone, UP WP1, the total lengths and bone areas of the incomplete NHMUK 39411 and 41637 were estimated by scaling these to the same external outline. The presence of substantial amounts of trabecular bone at the end of NHMUK 41637 suggests the bone is more complete, while NHMUK 39411 has little evidence of trabeculae and therefore is less complete. While there is some evidence of allometric scaling in the wing bones of pterosaurs (Witton pers. comm.), it is not well supported and does not significantly affect the conclusions drawn in this paper. Therefore, isometric scaling as described above was used. Once the total length was estimated and the fraction of missing bone was known, the area curves (Fig. 2) were extrapolated for each missing section. Each extrapolated point was determined based on the scaled position of the corresponding UP WP1 point. This was done until the total estimated length was reached. Once bone cross-sectional area was calculated for each bone, numerical integration of that area, using the trapezium rule, produced a total volume for each bone. To calculate bone mass, the bone volume was multiplied by a density of 2.05 g/cm^3 , the density of both bird and bat humeri (Dumont, 2010).

RESULTS

The length vs. area curves for each phalanx show that the general distribution of cross-sectional area in each bone follows a similar pattern (Fig. 2). In regions with large amounts of trabeculae (e.g., the ends of the bone), the cross-sectional area is larger, with more variability than through much of the shaft. The greater variability in the ends of the bone is possibly the result of the manual application of the software's 'Threshold' function. However, by integrating each curve, this noise is averaged in the volume and mass estimates (Table 2). To investigate the sensitivity of the estimate to the chosen threshold value, UP WP1 was re-analyzed using a constant value in place of per-slice manually-determined values. We found a variation in estimated volume of less than 2% (see Supplementary Material for more detail).

DISCUSSION

Our results show first wing phalanx masses that are 2.0 to 2.1-times greater than those found using Witton's (2008) method. This difference can be explained by two main reasons: (1) Witton's methodology does not account for trabeculae or the flaring of the section at the ends of the bones; and (2) he assumed a uniform cortical thickness, while our CT scans reveal significant thickness variation along the bone, and a greater average cortical thickness (Table 2).

Our approach offers a more accurate method for estimating the volume (and hence mass) of ornithocheirid pterosaur bones, and yields substantially higher values than those obtained via simple geometrical approximations. Consequently, if

these data are used as input to Witton's (2008) mass estimation technique, the body mass estimates are increased by roughly a factor of two, implying for example a 500 kg *Quetzalcoatlus northropi*. It may also be possible that the difference only applies to wing bones and that Witton's geometric methodology and cortical thickness regression would give similar results for the remainder of the skeleton. However, since wing mass may be almost 40% of the total body mass (Strang, 2009) the difference in body mass estimates would still be large (40%).

Clearly this methodology relies not only on skeletal mass estimation but also on the application of the relationship found in Prange et al. (1979) between skeletal and body mass in birds. However, recent work suggests that while birds may appear to be close living analogues to pterosaurs, they have a significantly different skeletal morphology, and degree of skeletal pneumatization (Witton and Habib, 2010), not to mention membrane flight surfaces and their total lack of feathers. Additionally, the relationship between skeletal mass and total mass is not consistent when expanded to snakes (Prange and Christman, 1976) and lizards (Metzger and Herrel, 2006). Furthermore, our ongoing analysis of data from Prange et al. (1979) indicates that the data has substantial scatter so may not be as useful as previously thought as a basis for mass estimation of specific animals that are distinct from demonstrating a general trend. Consequently, the skeletal-to-body-mass relationship found in birds may not be reliable for pterosaurs and the data scatter allows a range of possible values, so new techniques which combine body volume estimates and skeletal mass are required in order to improve mass estimation accuracy further. This could be achieved by a hybrid method that uses CT scans to determine skeletal mass,

combined with the whole body volume approach, to derive soft tissue mass, informed by the available pterosaurian muscular reconstructions, especially with regards to the massive volant forelimbs (e.g., Bennett 2003, 2008). This will allow for a more in-depth analysis of skeletal and soft tissue mass, and provide a better way of comparing these masses that is specific to pterosaurs.

In addition to pterosaurs, this method can be applied to other extinct animals, particularly those that possess a pneumatic skeleton. Postcranial pneumaticity is a key feature in extant birds (O'Connor, 2004), and has been recorded in numerous non-avian theropods (e.g., Benson et al., 2012) and basal avialans (e.g., Britt et al., 1998; Forster et al., 1998). It has also been widely displayed in the axial skeleton of sauropods (Wedel, 2007). Much like in pterosaurs, mass estimation in dinosaurs has proven challenging and several methods have been proposed (e.g., Henderson, 1999; Seebacher, 2001; Bates et al., 2009). This proposed method would assist in estimating the mass of pneumatized elements since it accounts for the varying amounts and distributions of air within a bone.

In conclusion, this new method for estimating pterosaur skeletal mass from CT scans implies body mass estimates (based on results for the first wing phalanx) twice as large as those obtained previously using a comparable methodology. If correct, this difference has considerable implications for pterosaur behavior and ecology. However, since this approach relies on a relationship established from extant birds, yet birds are questionable analogues for pterosaurs, the use of this CT method together with refinements to the alternative whole-body methods (through

total skeletal mass and soft tissue reconstructions) are a potentially better approach to further improving the accuracy of these mass estimates.

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FIGURE CAPTIONS

FIGURE 1. Representative sections without trabeculae and with trabeculae. **A, B**, NHMUK 41637 without trabeculae; **C, D**, UP WP1 with trabeculae. Images **A** and **C** are examples of unmodified CT scans, while **B** and **D** are modified images used for analysis with excess matrix removed and cortex reconstructed where necessary. Scale bar equals 5 mm. [intended for 2/3 page width]

FIGURE 2. Length vs. cross-sectional area plots. NHMUK 39411 (top), NHMUK41637 (middle) and UP WP1 (bottom). Outlines underneath are simple outlines of the bone in question. Solid outlines show portions of the bone that are present, while dashed outlines indicate the portion that is missing and total length has been estimated. [intended for single column width]

NHMUK 39411

NHMUK 41637

UP WP1