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Identifying Ranid urostyle, ilial and anomolous bones from a 15th century London well

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The accurate identification of bones from archaeological excavations is critical for the understanding of past faunas. In the United Kingdom, remains from East Anglian fens suggest that more anuran species existed in Saxon times than is the case today. Here, novel methods have been devised to determine the identity of anuran ilia and urostyle bones. These methods were used on remains from a 15th century archaeological site 200 metres north of St Paul's Cathedral, London, originally assumed to be common frog (*Rana temporaria*) with one possible water frog (*Pelophylax* sp.) imported as human food. The results suggest that the majority of the ca. 500 year old urostyle remains can be attributed to (in order of likelihood) *P. lessonae*, *R. arvalis* or *R. dalmatina*. The approaches described here complement existing methods and allow for more robust future identifications from zooarchaeological remains. A method is also suggested for taking the effect of growth on different parts of the same bone into account, thereby making bones of various sizes more comparable.

Key words: archaeological remains, bones, Britain, identification, ilia, *Pelophylax*, range, *Rana*, urostyle

INTRODUCTION

It has long been believed that Britain's amphibian fauna was impoverished as a result of the last glaciation and the subsequent interposition of the English Channel and North Sea between the British Isles and mainland Europe. For anurans, it has traditionally been assumed that, post-glacially, Britain only held the common frog (*Rana temporaria*), common toad (*Bufo bufo*) and natterjack toad (*Epidalia calamita*). However, subfossil remains consistent with pool frog (*Pelophylax lessonae*), moor frog (*R. arvalis*) and agile frog (*R. dalmatina*) in Saxon East Anglia have modified this view (Gleed-Owen, 1999, 2000). Furthermore, genetic and bioacoustic investigations have collectively provided robust evidence that pool frog populations, which became extinct in the UK approximately in 1994, represented relict populations from a former, more widespread native range (Zeisset and Beebee, 2001; Wycherley et al., 2002; Snell et al. 2005; Beebee et al. 2005). The former range of the pool frog is of particular interest as it is currently the subject of a reintroduction programme in Britain.

Here, frog skeletal remains excavated from a 15th century London site are examined to shed further light on the historical British anuran fauna. The anuran remains were recovered in 1979 by the Department of Urban Archaeology (Museum of London) from a former Greyfriars' convent garden well, approximately 200 metres north of St. Paul's Cathedral, west-central London (Ordnance Survey ref. TQ 320813 [Context 2033]). The amphibian remains from the excavation were originally described as likely belonging to at least six *R. temporaria*, with one urostyle offering the remote possibility of the earlier presence of pool or edible frogs (Clark, in Armitage et al., 1985), interpreted as discarded food items brought into London (Clark, pers. comm.). Details of the examined skeletal elements are shown in Fig. 1. A dashed ellipse surrounds the area known as the "corpus" at the posterior end of the Ilium. The ischium and pubis are rarely attached to the rest of the Ilium in finds at archaeological sites, and their original position is shown by outlines. The fracture plane of detachment (junctura ilioschiadica, j.i.) follows a line of weakness through the centre of the corpus (a posterior end view of the j.i. is also shown). The central, sub-circular concavity in the corpus – the acetabulum – is the articulation point for the femur; the T.S. (tuber superior) is a dorsal protuberance which can be species diagnostic (Böhme 1977, Böhme & Günther, 1979; Gleed-Owen & Joslin, 1996). In Fig. 1C an anterior end view of the urostyle is given. In lateral view, the urostyle is single shafted at the posterior end (right) but bifurcates into a double shaft at the anterior end; at termination these two shafts are capped by concave, sub-circular, bony surfaces (cotyles) which permit articulation with the rest of spine via the sacral vertebra.

MATERIALS AND METHODS

The author has kept the studied species in outside enclosures, and natural mortality of frogs provided a

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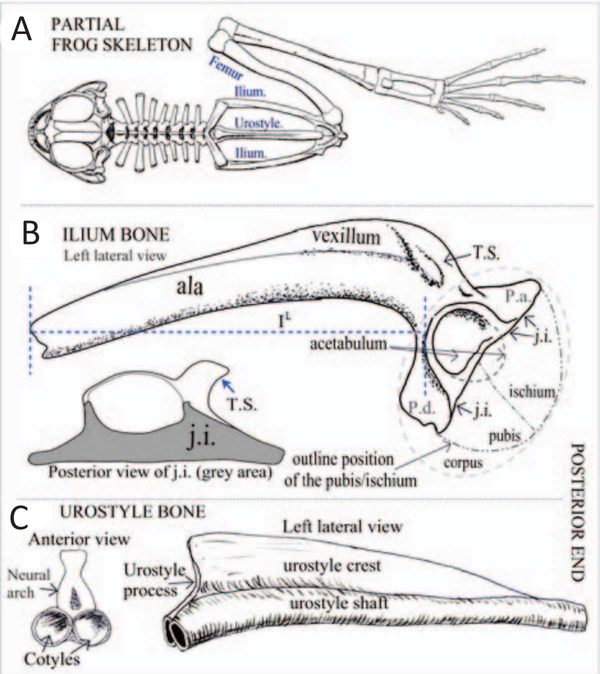


Fig. 1. The main bones discussed in this work. A) Position of the urostyle and ilia in a ranid skeleton (dorsal view, after Boulenger, 1897). B) Details of the left Ilium in lateral view and (lower left) posterior view. Abbreviations: T.S. = tuber superior, j.i. = junctura ilioschiadica, P.a. = pars ascendens, P.d. = pars descendens, IL = ilial length as defined in this work. C) Detail of a urostyle.

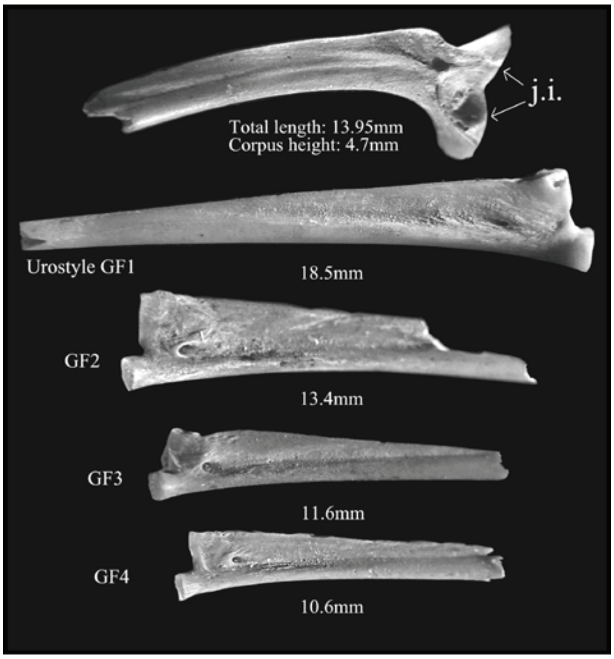


Fig. 2. Ranid bones from the Greyfriars' well site. Uppermost is a left Ilium, the rest (GF1-4) urostyles; GF1, right lateral view, GF2-4 left lateral. All lengths are given. All except the second bone from the top have been fractionally truncated (left-hand side of the ilium and right-hand tips of the lower 3 urostyles) due to damage at some point in their past.

reference bone collection. Mature snout-vent length ranges (SVL) for the species were obtained from the literature (Günther, 1990; Nöllert & Nöllert, 1992**;** Fog et al., 1997; Arnold & Ovenden, 2002).

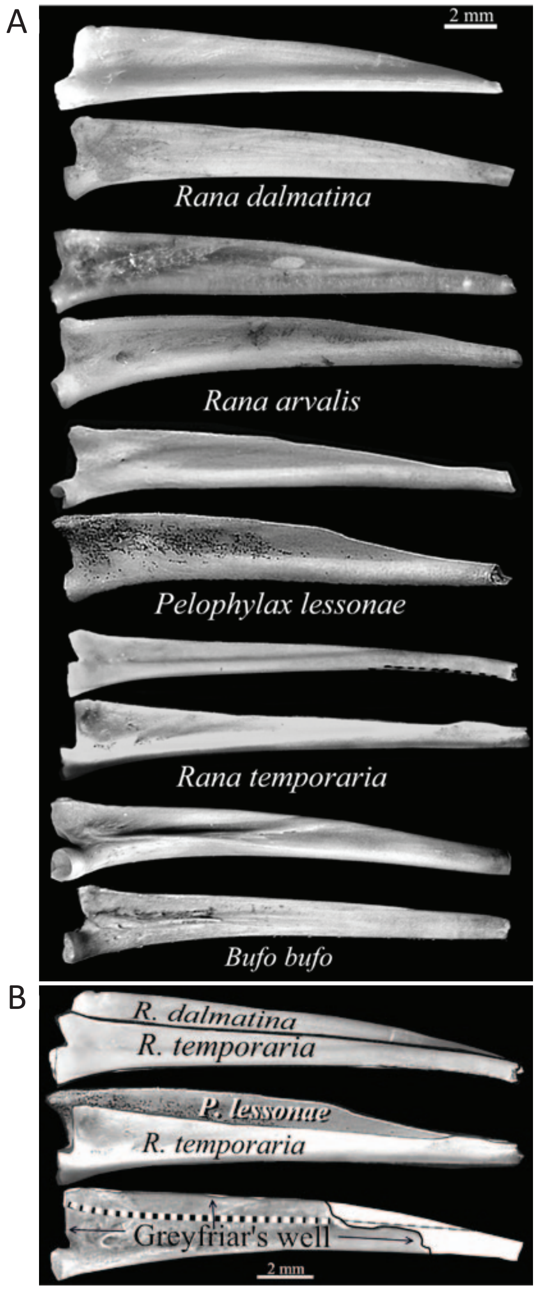


Fig. 3. A) Urostyle samples, two for each of the 3 NW European brown frogs, the pool frog (*P. lessonae*) and the common toad (*Bufo bufo*). B) Superimposed pairs of urostyle images. For each pair the lower bone surfaces were made level. Top: *R. dalmatina* urostyle overlain by the same length *R. temporaria* urostyle. Centre: *P. lessonae* overlain by a *R. temporaria* urostyle. Bottom: the Greyfriars' well urostyle (GF2) fragment (larger, grey portion at left) underlain by a modern urostyle similar in shape and size (an *R. arvalis* bone in this case, visible as white portion at right). This allowed an estimate of the original size of this subfossil. The black and white dotted line ca. 2/3 up from the ventral surface marks the upper height of an *R. temporaria* urostyle which was overlain, traced then removed.

To obtain measurements, images of frog urostyles and ilia, sourced from the collections of the author, the Natural History Museum (London), and C. Gleed-Owen, were enlarged 10–15 times and measurements were taken using a Vernier caliper or transparent ruler. Care was taken when photographing lateral views to ensure that the camera lens was perpendicular to the plane of the vexillum or urostyle crest and, for urostyles, that the resulting image only showed the shaft closest to the camera at the anterior, double-shafted end (Fig. 1C, Figs. 2–4). For ilial posterior view photography, the j.i. (Fig. 6) was positioned by eye to be parallel to the plane of the camera lens' front rim.

The main skeletal elements used for identifying anurans are the ilium, the frontoparietal, sphenethmoid and sacral vertebrae. Among the main osteological works depicting anuran skeletal elements, Rage (1974), Böhme (1977) Böhme & Günther (1979), Bailon (1999) and Ratinikov (2001) depict up to 1 ranid urostyle each, limiting interspecific comparisons. In this study, aspects of urostyles were photographed and tested for utility in species identification and contrasted with the line drawings of the same features in existing literature (Fig. 5A–B).

Sample numbers differed depending on the parameter investigated. The initial urostyle analysis was based on 10 *R. arvalis*, 12 *R. temporaria,* 20 *P. lessonae,* 6 *R. dalmatina*, 2 *B. bufo* and 4 sub-fossils from the Greyfriars' well (GF1-GF4). The initial Ilium samples amount to 16 *R. arvalis,* 14 *R. temporaria*, 23 *P. lessonae*, a single ilium from the well and 6 *R. dalmatina.* The *R. dalmatina* samples for both bone types included 5 specimens from France and one specimen from the Czech Republic. The Greyfriars' well sub-fossil urostyles and the single ilium are shown in Fig. 2.

Fig. 3A provides photographic lateral view examples of the urostyles used in this work, emphasising on the lower urostyle crest in *B. bufo* and *R. temporaria* in comparison to the other three species. The *B. bufo* urostyle samples also differed by possessing a lateral, protruding ridge – running for about half of the bone's anterior length (where the crest met the urostyle shaft). Fig. 3B shows urostyles overlain by those of *R. temporaria* for comparison. The dotted line in the lowest image marks the upper height of a *R. temporaria* urostyle which was first overlain then removed. The ventral sides of each pair are level with each other. The three truncated Greyfriars' well urostyles (GF2-4) had their images made semi-transparent using Microsoft PhotoDraw, then positioned and resized (maintaining image ratios) to fit over images of complete urostyles from the author's collection (e.g., Fig. 3B). The lowest depicted urostyle in Fig. 3B is GF2 which is underlain by the most closely matched which was aligned at the anterior (left) end. The method allowed later estimation of the original lengths of GF2-4.

As the length of the Greyfriars' fragmented urostyle remains (F) were known (Fig. 2), the original length of the complete bones (U) could be extrapolated using the equation $U=F[1+(B/A)]$, where A is the length (taken from the enlarged photographs) of a particular truncated urostyle, and B is the extra length added graphically (white section, right side of GF2 in Fig. 3B) to extrapolate original bone length. These extrapolations allow the later estimation of frog sizes.

Urostyle length (U^L) was measured from the anterior to the posterior tip. Proportional measurements of urostyle height (U^H) at specific points along the length of the main shaft (in lateral view) were also made using the enlarged photographs. For each urostyle, the diameter of the nearest-to-camera of the twin shafts was taken at the narrowest point at the anterior end, just before it merged into the urostyle process (Fig. 1C). The black bars in Fig. 4 represent these spans of the shaft diameter ($S^{\varnothing}$) and this measure was stepped out along the shaft starting at the position where the $S^{\varnothing}$ measurement was first made. For each bone, from a position equal to 4 and 8 horizontal spans of its $S^{\varnothing}$, vertical spans of this $S^{\varnothing}$ were stepped across U^H (Fig. 4); these measurements were rounded and recorded to the nearest 0.05 of a span. The procedure resulted in two U^H measurements for each bone in units of $S^{\varnothing}$ (Table 1).

Ilial length (I^L) was measured from the anterior tip of the shaft to the anterior edge of the acetabulum, shown as straight dashed lines in Fig. 1B (this acetabula edge is also shown as a curved dashed line in Fig. 7). This measurement was less affected by shape variation, the variable retention of the ischium and pubis, or damage at the corpus end (see also Esteban & Sanchiz, 1985). Using posterior views, measurements were made of acetabula diameter (distance from point D to D', Fig. 6), divided by bone thickness (T) at the centre of the acetabulum (D/T, following Böhme, 1977; Gleed-Owen, 2000). Fig.

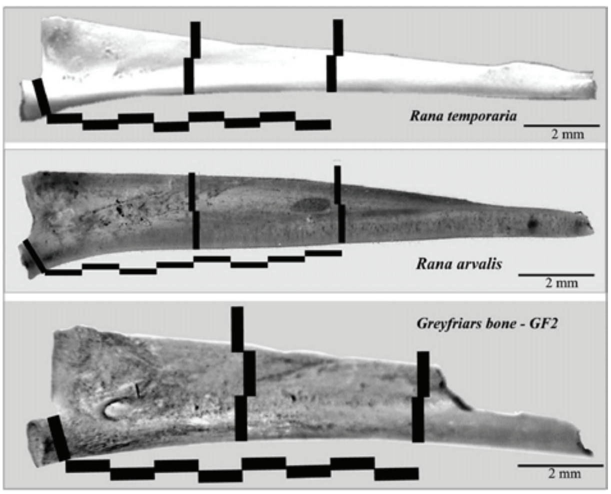
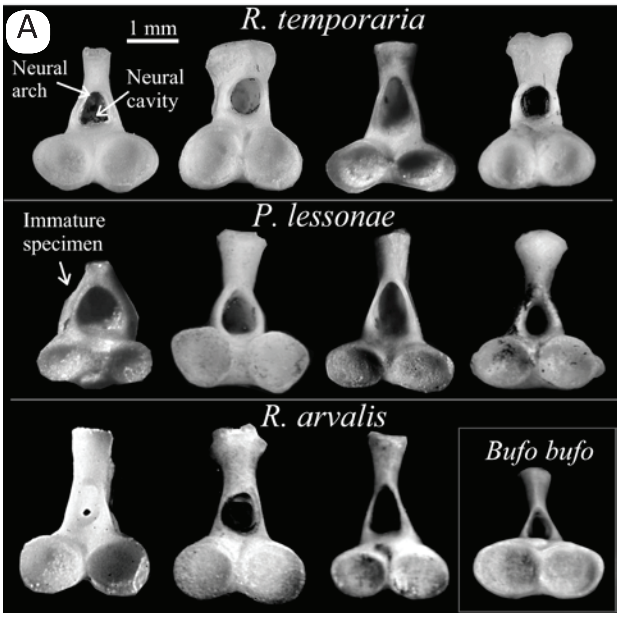


Fig. 4 (Left). Combined shaft and crest height (U^H) at fixed points along the urostyle. The black bars are spans of shaft diameter ($S^{\varnothing}$), taken at its narrowest point before it merges into the anterior process (somewhat diagonal black bars at far left). This was spanned along the urostyle to mark the 4 and 8 span positions. The vertical spans measure U^H in $S^{\varnothing}$ spans). Bone identification: top = *R. temporaria*, middle = *R. arvalis*, lower = Greyfriars' well urostyle GF2). Note the large difference between the *R. temporaria* urostyle crest height and the higher crests on the other species. This is emphasised by the span measurements (*R. temporaria* at 4 spans=1.65 (x $S^{\varnothing}$), at 8 spans=1.2; GF2 at 4 spans=2.2, at 8 spans=1.7).



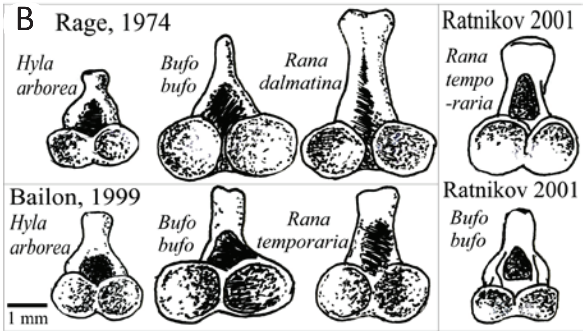


Fig. 5. Depictions of urostyle anterior views. A) Photographic views. The first two rows show a single species per row, the third row shows three examples of one species (*Rana arvalis*) followed by a single inset *Bufo bufo*. Supplementary information is given alongside the first specimen in the first two rows. B) Line drawings of urostyle anterior views, the first three specimens after Rage, 1974, the first three in the lower row after Bailon, 1999, and the right column after Ratnikov, 2001.

6C shows an accurate tracing of a *P. lessonae* ilium in posterior view in comparison to the single ilium from the Greyfriars' well (Fig. 6A-B). The thinner acetabula centre of 6A and B) suggests brown frog.

Further measurements were made of the combined height of the ilial shaft (ala) and the crest (vexillum) at specified points (b and c, Fig. 7) along the ilia using the distance between A-A' as a unit (distance A) to investigate interspecific height differences. Distance A represents the narrowest point of the bone in that region. Distance A-b and b-c on the ventral surface are reiterations of distance A. The vertical distances (labelled b-b' and c-c') were divided by distance A to obtain a relative measure of the combined height of the ala and vexillum (I^H). In this example, I^H at point c represents about 0.9 spans of distance A, and from point b approximately 1.1 times (Fig. 7).

The resulting measurements from urostyles and ilia were subjected to a series of two sample *t*-tests applied to permutated, two-species comparisons (Tables A1, A3 in the Online Appendix) before post-hoc, Holm's sequential Bonferroni testing (Holm, 1979).

To estimate any proportional change or trends in urostyle height in relation to urostyle length (U^L) as

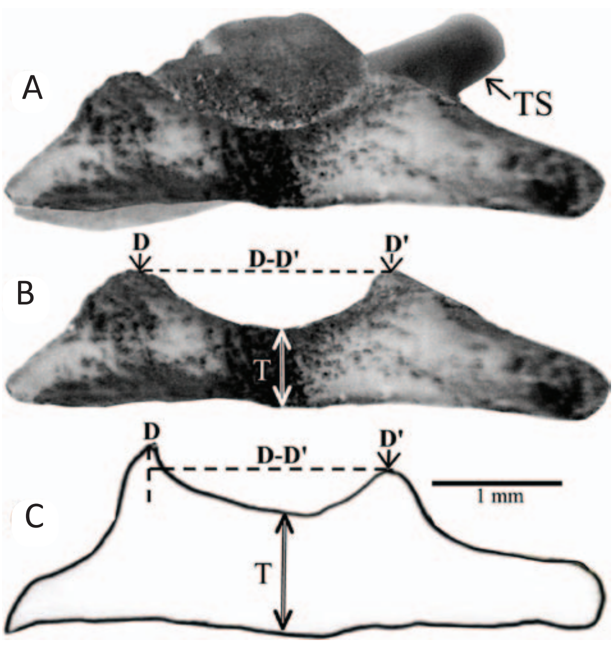


Fig. 6. Posterior end views of Ilia. A) shows the posterior end view across the junctura ilioschiadica (j.i.) of the Greyfriars' well ilium (TS=tuber superior) and B) shows only the junctura. Points D and D' are the outer edges of the acetabulum and D-D' represents its diameter at that point. Distance D (i.e. point D-D') divided by acetabula thickness at its centre (T) yields a ratio (D/T) which is somewhat group diagnostic (generally, brown frogs > water frogs). TS=tuber superior. C) shows a tracing of the j.i. in *P. lessonae*.

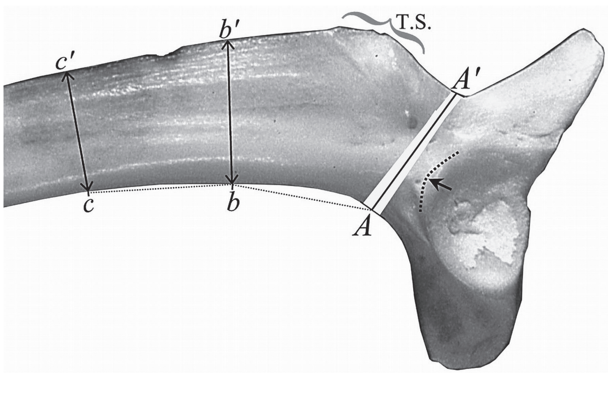


Fig. 7. Annotated photograph of the lateral posterior portion of an ilium. The white band running from A-A' represents the narrowest width of the bone at that point, the white band's width indicates the extent of sideways movement of this measurement when using callipers. The black line between points A to A' (distance A) represents the centre line of this white zone. Points b & c along the ventral surface are placed at reiterations of distance A. Lines c–c' and b–b' (placed perpendicular to the local ventral edge of the ilium) represent I^H extending from points c and b. T.S.=tuber superior. The anterior edge of the acetabulum (dotted line) is arrowed.

individuals grow, measurements of $U^H/S^{\o}$ were made at the 4 and 8 spans positions (Figs. 4 and 8) on a range of urostyles which allowed production of Fig. 9 (where U^H is denoted on the y-axis and U^L on the x-axis). The average positions for *x* and *y* are given as trend (regression) lines. These results were used to estimate the change (traditionally denoted by Delta (Δ)) in U^H (*y* -axis) per extra millimetre of growth in U^L. Here, the average change of *y* per unit *x* (i.e. $\Delta y/\Delta x$) was calculated using the equation $(y_2\text{-}y_1)/(x_2\text{-}x_1)=\Delta y/\Delta x$, where 1 represents the first measurement and 2 a subsequent measurement. The second readings use the average (trendline) parameters of more grown urostyles from the same species (Fig. 9). The change in U^H over a suitable length of the *x*-axis was used to increase measurement precision. As an example, of the method using Fig. 9C, an x-axis range of 15 mm was applied (here, 5–20 mm) and the corresponding U^H range found by projecting horizontally from the 5 and 20 mm points on the trendline to where they intercepted the y-axis and the two readings noted (for the 4S position this was 1.98 and 1.27 $S^{\o}$). The calculation (at 4S) is $\Delta y/\Delta x=(U^H_2–U^H_1)/(U^L_2–U^L_1)=(1.98\text{-}1.27)/(20\text{-}5)=0.71/15=0.047$. With $\Delta y/\Delta x$ calculated, urostyle height (U^H_2) at a given U^L (or vice versa) can then be derived from $U^H_1+(g(\Delta y/\Delta x))=U^H_2$, where g represents growth in mm. Expected U^H_2 after 3 mm of growth in U^L can be calculated as $U^H_1+(3(\Delta y/\Delta x))=U^H_2$. For each species, U^H and U^L averages were calculated for individuals within the estimated mature size range (Table A2; B , Online Appendix). U^L compensation could thus be applied to urostyles longer or shorter than average, for example when samples contain a large proportion of sub-adults. This was achieved using $\{(U^L_{Avg}\text{-}U^L_1)(\Delta y/\Delta x)\}+U^H_1=U^H_2$. Thereby, $\Delta U^H(=\Delta y)$ caused by U^L difference is compensated for (other individual divergences from the average $\Delta y/\Delta x$ are maintained) creating a clearer measure of U^H variation (e.g., Fig. 9D).

To estimate adult sizes for the Greyfriars' urostyles, similarity of bone morphology in the likely candidate species had to be considered. Three of the four Greyfriars' urostyles, although sub-adult, were already proportionally too high to be *R. temporaria*. Of the three species which had sufficient U^H proportions, insufficient data existed to evaluate *R. dalmatina* (only two U^L records). Combined *R. arvalis* and *P. lessonae* measurements were therefore used to assess mature size and proportions of these bones (Table A2; J). From the personal collections, five of the smallest frogs recorded at the onset of sexual maturity (three females and two males [which included one pair of *R. arvalis*]) had an average SVL of 52 mm (range, 44–56.5 mm). For the present investigation 50 mm was used as a measure of size at point of maturity (see also Arnold & Ovenden, 2002).

RESULTS

Apart from size differences, no discernible sex related differences in bone morphology were noticed for the two bone types examined here. Urostyle anterior ends varied significantly among specimens, however without apparent reliable species-diagnostic characters (Fig. 5). Therefore, identification using a single anterior view was not unambiguously possible. For example, the immature *P. lessonae* bone given in Fig. 5A might incorrectly suggest the genus *Hyla* on the basis of the first drawings in either row of 5B (both *H. arborea*). Bailon (1999) described the cotyles in *Hyla* as well differentiated from each other, although the first two *P. lessonae* (Fig. 5A) show greater separation of the cotyles compared to the *Hyla* drawings in Fig. 5B. The first bone in Fig. 5A shows the neural arch and neural cavity; features which display large variation in shape and size. The neural cavity can be circular or sub-triangular, with a range of sizes not necessarily related to the size of the bones. The shape of the urostyle process (Fig. 1) above the neural cavity also varied considerably, and cotyle shapes can be circular or elliptical. Urostyle anterior ends were not considered further here as useful in species separation. U^H measurements of the Greyfriars' unusually high crested urostyles (GF2-4) were not affected by the missing posterior pieces.

For *R. arvalis*, a positive correlation was suggested in $U^H/S^{\o}$ and the size of individuals (Table 1). Figs. 9B & C showed that a similar trend occurred in *P. lessonae* as well as *R. arvalis* i.e. that U^H relative to $S^{\o}$ tended to increase with extra U^L. Although this measurement was only taken for two *R. dalmatina* individuals, the longer urostyle had greater $U^H/S^{\o}$, suggesting the same could be true for this species. In *R. temporaria* the opposite is suggested by the data (Fig. 9A).

Table 1 gives individual U^H, average species U^H at 4 and 8 spans (4S & 8S). The Holm-Bonferroni tests applied more stringent alpha values to all *t*-test results, and only pair 4 at 4 spans (Table A1) was found to be non-significant. U^H at the 4S position was approximately $1^1/_3$ (avg. 1.37) times $S^{\o}$ for *R. temporaria,* and 2 x $S^{\o}$ (avg. 1.97) for *P. lessonae*. At 8S, U^H was $\leq$1 x $S^{\o}$ in *R. temporaria* (avg. 0.97) and approximately 1.5 x $S^{\o}$ in *P. lessonae* (avg. 1.54). The averages for *R. arvalis* and *R. dalmatina* at both positions were intermediate

U^H between *R. temporaria* and other species were significantly different from each other at 4S and 8S (Table A1). No significant separation existed between *R. dalmatina* and *R. arvalis* (p=0.890), *P. lessonae* and *R. arvalis* (p=0.117), and *R. dalmatina* and *P. lessonae* (p=0.1). Pairs 23 and 24 compare urostyles of *R. temporaria* with three sub-fossil urostyles (GF2-4) as a group (Table A1). Pair 23 is based on unmodified measurements while pair 24 used extrapolated average mature sizes (Fig. 8 and Table A2) for better comparison with bones from mature *R. temporaria*. The untreated measurements yielded *p* values of <0.0001 at the 4 and 8 spans positions (df=21). Allowance for maturation in the GF urostyles reduced the p values even further (p=<1 x10^{-8} at 4S and <1 x10^{-9} at 8S). GF 2, 3 and 4 were particularly distinct from *R. temporaria* at the 8 span position in respect of pairs 11, 15, 19, 23 and 24 in Table A1. GF1 most resembled *R. arvalis* at both positions and least resembled *P. lessonae*. GF2 and GF4 most resembled *P. lessonae* while GF3 resembled *R. arvalis* at 4S and *R. dalmatina* at 8S.

Table 1. UH/SØ values at the 4 and 8 spans positions. The results for *Rana arvalis* are arranged in order of urostyle lengths (longest uppermost), i.e. they range very approximately from the oldest to the youngest. A correlation can be seen in UH/SØ and the maturity of individuals. As the greyfriars' bones could represent more than one species, no averages are given. Key, N/A = not applicable, S = Spans, SD = standard deviation.

P. lessonae		*R. arvalis*		*R. temporaria*		Greyfriars		
4 S	8 S	4 S	8 S	4 S	8 S		4 S	8 S
2	1.8	2	1.7	1.1	0.7	GF1	1.6	1.15
1.8	1.2	2	1.6	1.2	0.95	GF2	2.2	1.7
2.1	1.7	1.75	1.45	1	0.8	GF3	1.75	1.25
2	1.6	1.65	1.3	1.4	1	GF4	1.9	1.65
1.95	1.75	1.75	1.5	1.6	1		Averages	
2	1.5	1.6	1.4	1.3	0.9		N/A	N/A
1.7	1.3	1.8	1.3	1.5	0.9			
2	1.3	1.6	1.2	1.5	1.05			
2.2	1.7	1.6	1.35	1.2	1			
2.2	2	1.6	1	1.6	1.2			
1.8	1.15	(In approx. age (descending) order)		1.5	1.1			
1.8	1.25			1.5	1.05			
2	1.5						*R. dalmatina*	
2.25	2						4 S	8 S
2.15	1.9						1.7	1.1
1.7	1.4						1.75	1.5
1.75	1.2						1.65	1.35
1.95	1.35						1.65	1.3
2.2	1.85						1.85	1.4
1.75	1.4						2.1	1.55
Averages (lower row (italics)=SD)								
1.97	1.54	1.74	1.38	1.37	0.97		1.78	1.34
±0.18	*0.28*	*0.16*	*0.19*	*0.2*	*0.13*		*0.17*	*0.15*

Figure. 8 visually reinforces *R. temporaria*'s distance from the other mature specimen plots, with U^H levels of *P. lessonae* being noticeably higher. Disregarding the immature proportioned *R. arvalis* plots, GF1 (a mature bone, U^L 18.5 mm) plotted amongst mature *R. temporaria* while GF2, GF3 and GF4 plotted distant from that species. GF2 plotted high into the *P. lessonae* grouping while GF3 and GF4 plotted among *P. lessonae, R. arvalis* and French *dalmatina*. The *R. dalmatina* from the Czech Republic plotted close to *P. lessonae. Bufo bufo* was included for comparison with the Greyfriars' bones but were most similar to *R. temporaria*.

A summary of urostyle data is given in Table A2; A–C, F, J and Table 2, while Table A2; D–E & G and Table 2 (central 3 columns) give the same for ilial parameters. Urostyle and ilial length and height statistics for all samples are given in Table A2; A–E (with separate results for mature individuals for both bone types). $\Delta y/\Delta x$ results are given in F & G. Table A2; H gives body lengths (SVL) as a proportion of U^L and I^L. A selection of abbreviations is given in Table A2; K.

Average SVL was approximately 3.6 times the urostyle or ilial length (Table A2; H), resulting in a minumum U^L of 13.97 mm and a minimum I^L of 14 mm (Table A2; B & E). Selecting only individuals within this limit forced a 50% reduction in the *R. arvalis* U^L total sample number (Table A2; A–B).

Fig. 9 and Table A2; F give the estimated average change ($\Delta y/\Delta x$) in U^H per mm growth in U^L. For *R. arvalis*, this was an additional 0.040 $S^{\varnothing}$ at 4S and 0.033 $S^{\varnothing}$ at 8S: the respective values for *P. lessonae* were 0.047 and 0.043. U^H change was, therefore, greater in *P. lessonae*. For *R. temporaria*, $\Delta y/\Delta x$ at 4S was 0.006 $S^{\varnothing}$ at 4S and -0.008 $S^{\varnothing}$ at 8S, indicating low $\Delta y/\Delta x$ in this species. Defining U^L_1 as the present urostyle length and U^L_2 as the calculated mature average for the species (15.95 mm for *R. arvalis* and 17.74 mm for *P. lessonae,* Table A2; B), while defining U^H_1 as the specimen's actual U^H, and incorporating $\Delta y/\Delta x$ increments to give U^H_2, leads to $U^H_2=U^H_1+((U^L_2-U^L_1)(\Delta y/\Delta x))$. Using the smallest *R. arvalis* in Fig. 8 (raw U^L=8 mm, U^H=1.6 $S^{\varnothing}$ at 4S and 1.0 $S^{\varnothing}$ at 8S), for example, leads to the calculation; U^H_2=1.6 + ((15.95-8)(0.04))=1.6+(7.95 x 0.04)=1.92 $S^{\varnothing}$ at 4S

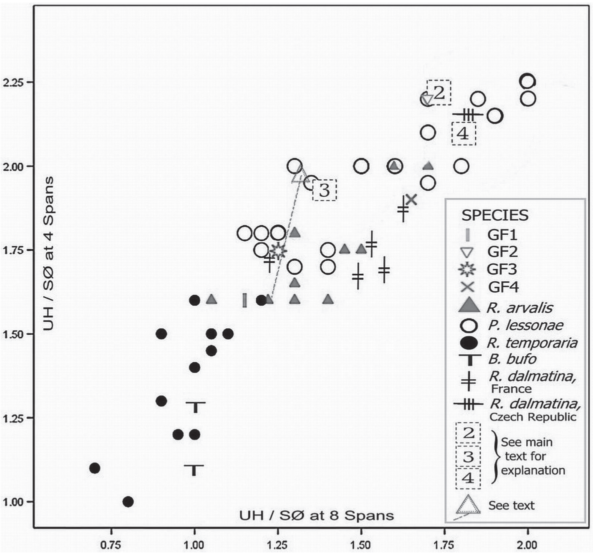


Fig. 8. Scatter plot of U^H of NW European frog species and *B. bufo* expressed as $U^H/S^{\varnothing}$ (U^H divided by shaft diameter). The y-axis shows U^H at the 4 spans position, and the x-axis U^H at 8 spans. While the *R. arvalis* results showed overlap between the other three species, good separation resulted between the *R. temporaria*, (French) *R. dalmatina* and *P. lessonae*. The small overlap between the *R. temporaria* and the more juvenile (lower plotting) *R. arvalis* is consistent with the relationship between age/size and relative U^H. Greyfriars' urostyle 1 (GF1) plotted between *R. temporaria* and the younger *R. arvalis*. The *B. bufo* samples were closest to *R. temporaria*. The three boxed numbers (2, 3 & 4 at top right) represent GF2, 3 & 4, respectively, after allowance for maturation (Fig. 9A-C, Online Appendix Table A2F & A2J). The open triangle between the two high *R. arvalis* plots is a mature size extrapolation applied to the smallest *R. arvalis* urostyle (linked by dotted line).

and, similarly, U^H_2=1.0+(7.95x0.033)=1.0+0.26=1.26 $S^{\varnothing}$ at 8S.

These treatments allowed a re-plot of this specimen (open triangle, Fig. 8), where it is joined to its original position (solid triangle) by a dotted line. Fig. 9D shows plots for 12 *R. arvalis* samples both treated and untreated for comparison, alongside unadjusted *R. temporaria*. Separation was clearer when the varying degrees of maturity in the *R. arvalis* bones was compensated for. The results also illustrate the effect of U^L/U^H and suggest that length standardisation is of use for species identification.

The calculations in Table A2; J focus on GF2-4 and use the calculated combined (*lessonae-arvalis*) average mature U^L of 17.46 mm (C). The estimated lengths of Greyfriars' urostyles gave a pre-fracture U^L of 16.75 mm for GF2 and 13.4 mm post-fracture; for GF3, pre- 13.2 mm and 11.6 mm post-; GF4, 12.67 mm pre- and 10.6 mm post-fracture (Table A2; J). Estimations of mature U^H for GF2-4 were also made using data from Figs. 9A–C and Table A2; C, F & J. For example, GF3 needed an extra 4.31 mm to reach average U^L (Table A2; J, row 3) and this was multiplied by the relevant $\Delta y/\Delta x$ increments (Table A2; F). The calculations here were made conservatively using the smaller $\Delta y/\Delta x$ amounts of *R. arvalis* compared to *P. lessonae* at both 4S and 8S (e.g.

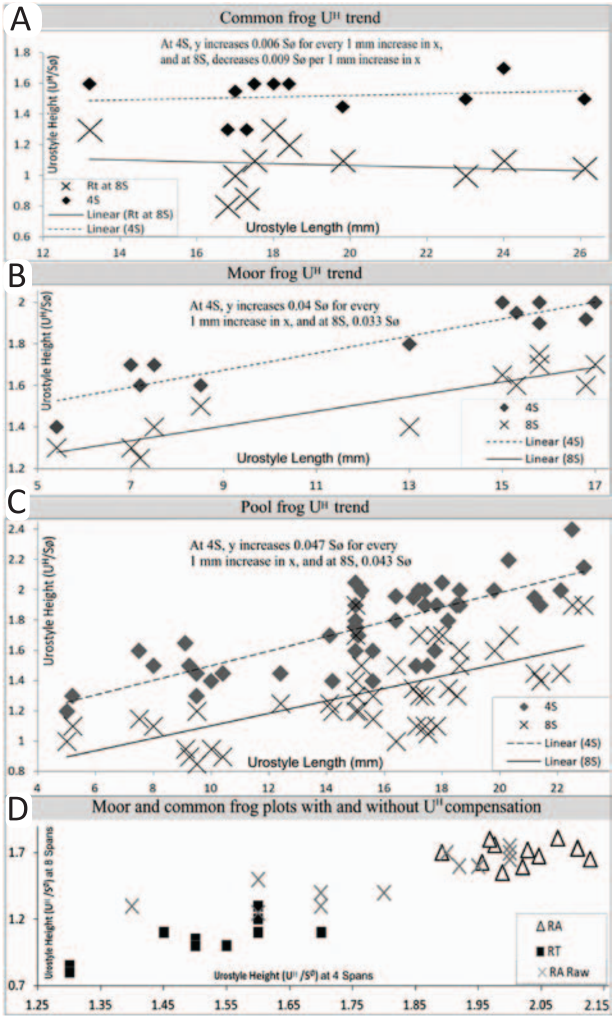


Fig. 9. A–C) Scatter plots of urostyle length (x-axis, in mm) and Urostyle height (y-axis) as $U^H/S^{\varnothing}$). 9A shows *R. temporaria* (RT), 9B, *R. arvalis* (RA) and 9C, *P. lessonae* plots; U^H at 4 spans (4S) are shown as black squares and 8S plots as crosses. The two slopes in each of A–C are computer generated regression (trend) lines for the respective species. From these lines, the amount of extra U^H (y-axis) gained with additional length (x-axis) can be read, and from this the U^H change per millimetre of growth in U^L ($\Delta y/\Delta x$) can be calculated. These amounts – in text – are given in the respective plots for each species. D) shows U^H at 4S (x-axis) and at 8S (y-axis). The black squares represent *R. temporaria*, the grey crosses *R. arvalis* in a range of sizes as originally found and the triangles are *R. arvalis* results after mathematical compensations were applied to nullify U^H differences arising from size/age differences. This has led to a clear separation between the two species not present before compensation.

0.04 $S^{\varnothing}$ and 0.033 $S^{\varnothing}$ respectively). The U^H amount required to reach adult U^H was calculated. For GF3, for example, this was 0.172 $S^{\varnothing}$ at 4S (4.31 x 0.04 $S^{\varnothing}$) giving a total U^H at 4S of 1.92 $S^{\varnothing}$ (0.172 + original U^H of 1.75 $S^{\varnothing}$ (Table A2; J [row 4]). The equivalent at 8S was 1.39 $S^{\varnothing}$.

Amended plots, which account for maturity in GF2-4 (using the *R. arvalis* figures for 4S and 8S) are given in Fig. 8. In this analysis, the Greyfriars' urostyle GF2 is little

Table 2. Summary of 2 sample *t*-tests. Rows 1–6 compare species pair-wise, rows 7–10 compare the Greyfriars' ilium with those of identified species. Column b-b' and c-c' compare I^H results from points "b" and "c" and heavy black crosses indicate best match for the GF ilium. All except D/T are novel measures. The larger solid spots indicate extremely high statistical significance ($p<0.0005$), hollow spots=highly significant ($p<0.005$) and the smaller black spots=significant separation ($p=\leq 0.05$). x=no statistical significance ($p>0.05$).

KEY: ● =p<0.0005, ⊙ p<0.005, • p ≤ 0.05, X =p>0.05, ✚=closest match to GF ilium, cf=compare, n/a=not applicable, sp.=span.

Species or individual	Row No.	ILIA			UROSTYLES		
		ilial height		corpus	height		
		Measure of sample statistical separation at position :					
		b-b'	c-c'	D/T	4-Sp.	8-Spans	4+ 8sp
R. temporaria cf. P. lessonae	1	●	●	●	●	●	●
P. lessonae cf. R. arvalis	2	X	•	●	⊙	X	•
R. temporaria cf. R. arvalis	3	●	●	X	●	●	●
R. dalmatina cf. P. lessonae	4	•	●	●	•	X	X
R. dalmatina cf. R. temporaria	5	●	●	X	●	●	●
R. dalmatina cf. R. arvalis	6	X	●	X	X	X	X
Greyfriars ilium cf. R. temp.	7	✚	✚	✚	n/a	n/a	n/a
Greyfriars ilium cf. R. arvalis	8	⊙	X	X	n/a	n/a	n/a
Greyfriars ilium.cf. P. lessonae	9	⊙	X	●	n/a	n/a	n/a
Greyfriars ilium cf R. dalmatina	10	•	•	n/a	n/a	n/a	n/a

changed but GF3 and GF4 are even more distant from *R. temporaria*. GF2 and GF4 both plot high into the near exclusive range for *P. lessonae* with GF3 plotting within the ranges of *R. arvalis* and *P. lessonae.* GF1 was already at a mature size and therefore had no plot adjustment. In Figs. 9A–C, U^H increments per mm U^L increase ($\Delta y/\Delta x$) are given. These show that the *R. temporaria* samples had a calculated per mm increase in U^H of 0.006 $S^{\varnothing}$ at 4S and a decrease of 0.009 $S^{\varnothing}$ at 8S. *R. arvalis* (Fig. 9B) had a gain of 0.04 $S^{\varnothing}$ per mm at 4S and 0.033 $S^{\varnothing}$ at 8S; and in *P. lessonae* (9C) the results were 0.047 $S^{\varnothing}$ at 4S and 0.043 $S^{\varnothing}$ at 8S. The average of the last two species means was 0.044 $S^{\varnothing}$ (4S) and 0.038 $S^{\varnothing}$ (8S).

Ilial height proportions enabled partial separation of the frog species (Fig. 10). Separation of *R. temporaria* is good and, as with the urostyle results, the wider range of bone sizes for *R. arvalis* is apparent. Fig. 10 suggests that ilial height measurements at point b could be useful for non-specialists to quickly determine if a bone represents *R. temporaria*.

Gleed-Owen (2000) lists D/T ratios between 2.75 and 4.00 in brown frogs and 2.12–2.88 in water frogs (note a zone of overlap from 2.75–2.88). In the present study, D/T only separated *R. temporaria* from *P. lessonae* (Fig. 11), whereas *R. arvalis* overlapped with *P. lessonae and R. dalmatina*. Measurements of I^H suggest a separation of *R. temporaria*, *P. lessonae* and, given more data, possibly *R. dalmatina* (Fig. 11). The *t*-tests (Table A3) suggest that I^H in *R. temporaria* is clearly separable from the other species means at point b (b-b') and point c, where *P. lessonae* was distinguished from *R. dalmatina*. The Greyfriars' well single ilium was closer to *R. temporaria* at points b and c ($p=0.47$ and 0.79 respectively). The D/T *t*-tests results (no dedicated Table given) showed that *P. lessonae* was well separated statistically ($p<0.005$) from the other species. The Greyfriars' well ilium's D/T result was closest to *R. temporaria (p*=0.850), followed by *R. arvalis* ($p=0.480$) and considerably separate ($p<0.005$) from *P. lessonae*. The single *R. dalmatina* D/T result was intermediate to *R. temporaria* and *R. arvalis*.

A summary of results on the urostyle and Ilial analysis (including D/T) is provided in Table 2. Comparing ilial parameters (b-b, c-c and D/T) it shows that c-c' (I^H at point c) is the most discriminating between the four species. The *R. temporaria* urostyles and Ilia are distinct from *P. lessonae* means in all measures. *P. lessonae* was best separated from *R. arvalis* using D/T and U^H at 4S. *R. temporaria* was distinguishable from *R. arvalis* and *R. dalmatina* in all parameters except D/T. The *R. dalmatina* and *P. lessonae* were separable, particularly in I^H at point c and D/T; their U^H at 4 spans was also significantly separable ($p\leq 0.05$). The *R. dalmatina* and *R. arvalis* were

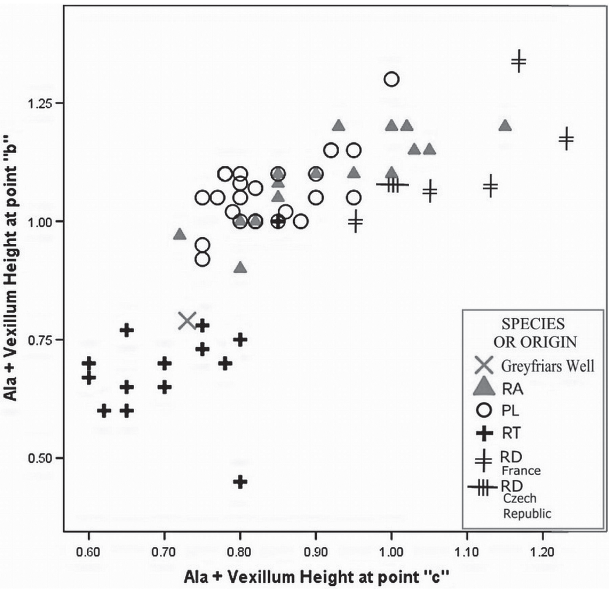


Fig. 10. Diagnosing species using I^H divided by distance A (Fig. 7). (Abbreviations: RA=*R. arvalis*, RT=*R. temporaria*, RD=*R. dalmatina*, PL=*P. lessonae,* I^H=ilial height (ala+vexillum combined). Ilial heights were measured from points b and c using distance A as a unit (Fig. 7); the results are displayed here (y-axis and x-axis respectively). This parameter was relatively easy to record using enlarged photographs or scans and produced varying separation of the species, being particularly good for *R. temporaria* at point b. The Greyfriars' Ilium (diagonal cross) has grouped with the *R. temporaria* results.

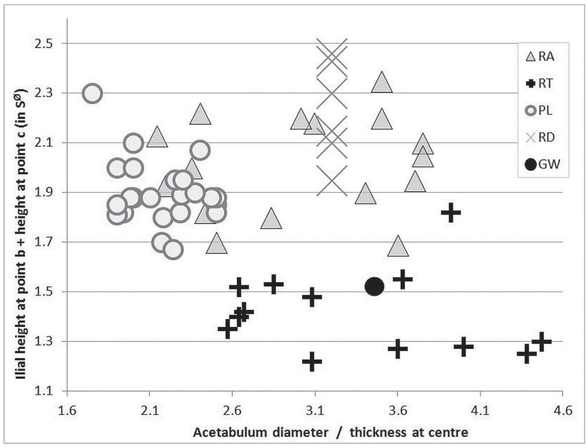


Fig. 11. I^H at points b and c plotted against D/T. This plot shows varying separation of the species. Separation of *R. temporaria* was better using I^H as opposed to D/T and the reverse for *P. lessonae*. The Greyfriars' well ilium (black spot, D/T=3.46) plotted among the *R. temporaria* results. The linear nature of the *R. dalmatina* results is explained in the text. (Abbreviations: RA=*R. arvalis*, RT=*R. temporaria*, PL=*P. lessonae*, RD=*R. dalmatina,* GW=Greyfriars' Well ilium).

best distinguished using I^H at c-c' while the Greyfriars' ilium most resembled *R. temporaria* in all measures. Comparing the relative species separation utility of both I^H and D/T (Table A2, Table 2), it can be seen that I^H (at point c) was significant in pairings with *R. temporaria*; similar high significance ($p<0.0005$) occurred in the *dalmatina*/*lessonae* pairing and $p=\leq 0.05$ for the *arvalis*/*lessonae* pairing.

Because of the clear distinction between the *R. temporaria* and GF2–4 urostyle results and the paucity of data for *R. dalmatina*, it seemed pertinent to consider only *R. arvalis* and *P. lessonae* samples when comparing bone sizes and the expected size of the GF2–4 frogs.

Using the ratio SVL/U^L=3.58 (Table A2; H) with the extrapolated sizes for the Greyfriars' urostyles (4J) gave the following: GF2 *in vivo* body length 60 mm; GF3, 47 mm and GF4, 45 mm. An average adult *R. temporaria* has an SVL of about 80 mm (Nöllert, 1992), and edible frogs (*P. esculentus*) have an average SVL of approximately 100 mm (Günther, 1990; Nöllert, 1992).

Fig. 12 correlates body length with an estimation of expected mass. The combined (*P. lessonae* and *R. arvalis*) average U^L of 17.46 mm (Table A2; C) suggests an average body mass of approximately 62.5 g and, extending the range shown, a 100 mm edible frog would approximate to 107 g. This suggests that the Greyfriars' urostyle GF2 is derived from a frog of about 22 g while GF3 and GF4 is derived from a frogs of less than 15 g, rendering the edible portion of the Greyfriars' frogs (about 1/3rd) negligible.

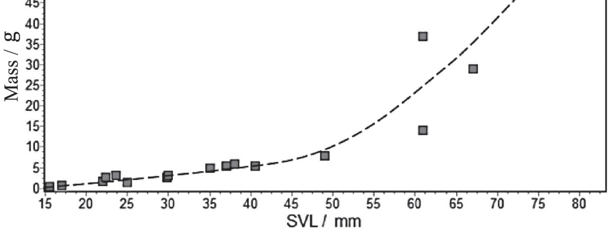


Fig. 12. The relationship of body length and frog mass. The graph indicates that body mass increase accelerates after the frogs reached an SVL of 50 mm.

DISCUSSION

The novel methods described here allow quantitative analyses. The novel ilial method, used alone or in combination with the previously established method (D/T), was useful in separating *R. temporaria* from other brown frogs and *P. lessonae*. The novel use of urostyle ratios also separated *R. temporaria* from the other species. This attribute is particularly useful as *R. temporaria* is the main frog species expected in archaeological excavations in the UK, and urostyles have the advantage of regularly being preserved.

The presented analyses did not support the contention that all of the Greyfriars' ranid remains were derived from *R. temporaria.* Of the five sub-fossil bones under investigation, three out of four Greyfriars' urostyles showed the least similarity to *R. temporaria*, in contrast to Armitage et al. (1985). The statistical results show that the likelihood that GF2–4 (collectively) formed part of the *R. temporaria* population is highly unlikely. Urostyle GF1 was ambivalent (occasionally showing a closeness to *R. arvalis*) but if the immature *R. arvalis* samples were discounted, it more resembled *R. temporaria* in agreement with Gleed-Owen and Clark (pers. comm.).

A changing species specific relationship between U^H and U^L (relative to $S^Ø$), with increasing maturity was identified. This allowed extrapolation of mature bone sizes from juvenile proportions, leading to less ambiguous species separation. The changes in bone proportions described here suggest that a fair test for species separation would need either size compensation treatments or samples of similar maturity. That such samples are rarely available confirms the utility of the approach presented here to quantify inter- and intra-specific variation and growth related change. Future research, especially involving *R. dalmatina* and *R. arvalis,* would benefit from higher sample numbers.

The small, immature sizes of most of the frog bones in the Greyfriars' well and their presence alongside small mammal bones, increases the probability that they were natural casualties rather than discarded food items. It may also be pertinent that the Greyfriars were a relatively frugal and devout Franciscan order who should have been aware of biblical warnings on the eating of certain "unclean" meats, including frogs ("unclean spirits", Revelation, 16:13, "all that move in the water that do not have fins and scales...they are an abomination to you", Leviticus, 11: 4–8, 10–19, 24–30).

The results strongly support a loss of anuran diversity and a local assemblage from the 15th century in which *R. temporaria* was possibly not the most common species. *Rana temporaria* benefits more from man-made elements in the environment (e.g., garden and ornamental ponds, Carrier & Beebee, 2003) than the other species found. *Pelophylax lessonae* populations need numerous and varied water bodies spread over a larger area to assure continuous occupancy (Sjögren, 1988) which might not have been locally available when the marshy areas around the Thames were drained. The findings of Gleed-Owen (2000) suggesting the existence of *R. temporaria* and three other species in Saxon East Anglia, infers that London could still have had additional species in the 15th century. Roček and Šandera (2008) suggest that the absence of *R. arvalis* in southern and western Europe (including Britain) could be explained by recent deforestation and drainage. Given its current distribution, the presence of *R. dalmatina* in London in the 15th century would appear the least likely, although a more extensive range which included the UK in middle Saxon times (ca. 600–950 AD) has been previously inferred (Gleed-Owen, 2000). The evidence suggested here extends forward the possible existence in the UK of frog species, other than the common frog, by at least 500 years.

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