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Research Article

Separating brown and water frogs to group & species on snout features

CHARLES A. SNELL

27 Clock House Road, Beckenham, Kent BR3 4JS

ecofrog@bigfoot.com

ABSTRACT - Identification guidance in the herpetological literature for distinguishing between the northwest European brown frog species and brown frog and water frog groups (*Rana* and *Pelophylax* respectively) often describes the degree of snout pointedness as a diagnostic feature. This method is evaluated here and is found to have no value. On the other hand, a novel method, using the markings on the snout, has value in separating the north-west European brown frogs and in separating the two groups. Consideration is given to the possibility that earlier descriptions of species having more pointed heads than others is due to variation in the narrowness of the angles of head markings causing an illusion of greater head pointedness.

INTRODUCTION

This work has a main focus on the six indigenous northwest European frog species, comprising three brown frogs, the Common Frog *Rana temporaria*, the Moor Frog *Rana arvalis* and the Agile Frog *Rana dalmatina*, and three water frogs, comprising two species, the Pool Frog *Pelophylax lessonae* (formerly *Rana lessonae*), and the Marsh Frog *Pelophylax ridibundus* (formerly *Rana ridibunda*) and their hybrid, the Edible Frog *Pelophylax* kl. *esculentus* (formerly *Rana* kl. *esculenta*).

Water frogs are often described as having a more pointed snout than brown frogs (e.g. Wycherley, 2003; Natural England, 2008; Arkive-Images of Life on Earth, 2011). In Fig. 1 (from Arnold & Ovenden, 2002) the water frog is depicted with a more pointed snout than the brown frog and Inns (2009) describes the Common Frog as having a blunter snout than water frogs. Additionally, within the brown frogs, Common Frogs are often described as having a less pointed snout than the Moor Frog (e.g. Haltenorth, 1979; Chihar & Cepika, 1979; Hofer, 1985; Laňka & Vít, 1989; Nöllert & Nöllert, 1992) or usually having a less pointed snout (Fog et al., 1997), Arnold and Ovenden (2002). The ubiquity and longevity of this distinction is highlighted by the fact that in Scandinavia the Moor Frog is called the Pointed Nosed Frog (spissnutefrosk (Norwegian), spidssnudet frø (Danish)) whereas the Common Frog is known as the Blunt-nosed Frog (buttsnutefrosk (Nor.), butsnudet frø (Dan)). The usefulness of using these characteristics for distinguishing between species or groups is evaluated here.

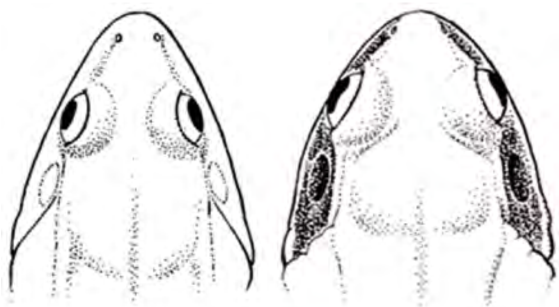

Figure 1. Depiction of the water frog snout (left) compared to brown frog (right). From Arnold & Ovenden, 2002.

It was noticed in the course of the work on snout form, eye stripes and eye shape, that the dark facial stripes that pass across each nostril and join to the anterior edge of each eye, had quite varying angles; the utility of these are also investigated.

METHODS AND MATERIALS

Samples available for comparison

Species and sample numbers of north-west European frogs used for the investigation were as follows: water frogs, 27 (of which Pool Frogs, 15, Edible Frogs 5, Marsh Frogs 7), Common Frogs 33, Moor Frogs 19 and Agile Frogs 14.

Measuring snout pointedness

To quantify snout pointedness, electronically cut-out photographs of the heads (photographed vertically from above) of a range of water and brown frog individuals were made (Fig. 2); no conscious bias was made in the selection. These were transformed into black silhouettes (water frogs) or white silhouettes (brown frogs) and then overlain in pairs (example shown in Fig. 2B) to check for consistent variation in snout form (approximately the upper one third of the image) between the brown and water frogs. A less time-consuming method was adopted part-way through the investigation, where a line was traced - using graphical software (MS PhotoDraw) - around the head region starting and terminating at the points where the arms met the body (Fig. 2C). These tracings overlain in permutated pairs were then individually ordered in terms of snout pointedness.

In both methods the lower two thirds of the outlines were aligned and sized (conserving proportions) to match each other as closely as possible; a method which also removed differences brought about by the size of the individual.

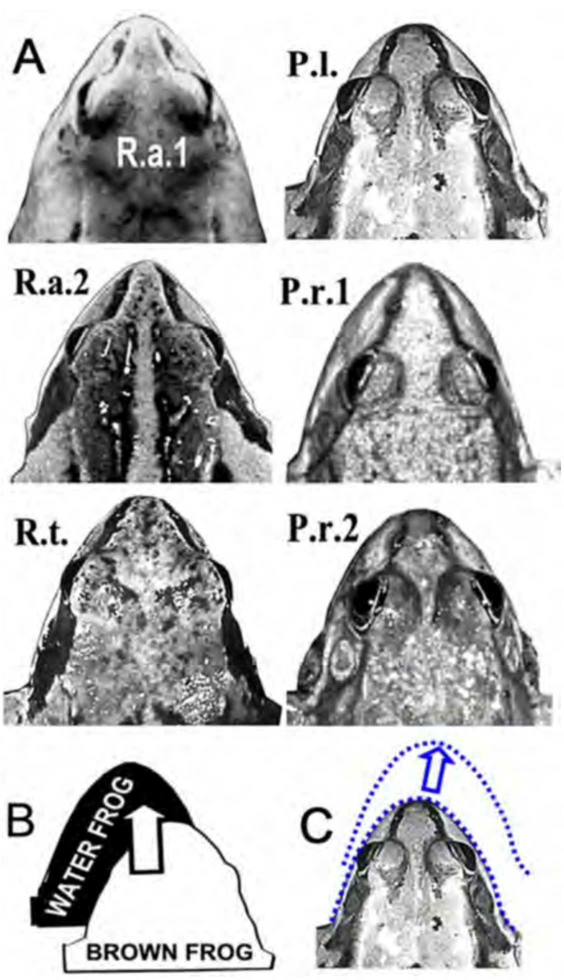

Figure 2. Electronic cut-out photographs of the heads of a range of water and brown frogs.

Facial markings and angles

The possibility of separating the groups - or even species - by differences in facial markings, proportions or angles was investigated by creating a series of computer generated tracings taken from enlarged photographs. These traced the eyes as well as the dark stripes which, starting near the snout tip, pass through the nostrils and stop at the anterior part of the eye. Fig. 3 gives an example of these and the position of tracing is shown at B, where an outline of the head is included for clarity. The eyes are the sub-semicircular shapes in the lower part of each traced line.

The angles between the two stripes - estimated by best fit (see grey dotted lines in R.a.′, Fig. 3) - were recorded for these and the other specimens in the samples: the turns in the facial markings anterior to the nostrils (e.g. in R.a′ and R.d) and the more pronounced turns closer to the eyes (e.g. see arrows in R.a, R.a.′ and R.d. specimens, Fig. 3) were excluded from these “best fit” considerations.

Clearly, the greater the proportion of width compared to length, the greater the amount of eye visible from above and the more upwardly focused the frog eyes are (q.v. Snell, 2011). The right eye of Pr′ shows the approximate placing of eye measurements (L = length, W = width) later used to estimate the amount of eye visible from above. The dotted lines in the R.d. and P.l. specimens were aligned to the dorsal edge of the eye and illustrate a difference between water and brown frogs in these alignments; they were not used for the angle measurements.

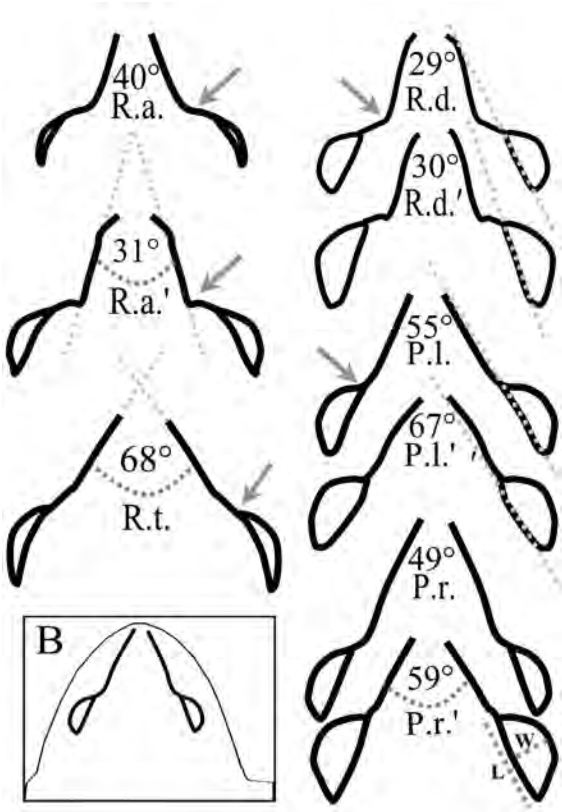

Figure 3. Computer generated tracings and estimated angles (dotted grey lines).

Statistics
Measurements of facial stripe angles and eye proportions were subject to ANOVA tests; these were followed by 2-sample t-tests to pinpoint where variation lay. The t-test results were subject to sequential Bonferroni testing (Holm, 1979); this placed more stringency on the rejection of the null hypothesis.

RESULTS

Snout pointedness - brown frogs and water frogs
Fig. 4 shows a representative sample of the possible combinations produced from the black silhouettes (water frogs) overlain with the white silhouettes (brown frogs). In total 27 water frogs and 66 brown frogs were used. The result, as with the sample in Fig. 4, showed little difference in pointedness. An observer in the field would be ill-advised to base identification on snout pointedness. The top one third (the snout area) of silhouette pair "B" (Fig. 4) shows that this Marsh Frog has a slightly more pointed snout than the Common Frog. None of the other silhouettes or traced outlines showed substantive differences between water and brown frogs.

Snout pointedness – brown frog results
Of the brown frogs (N = 66), the three most pointed head outlines (4.5%) belonged to Moor Frogs; the five least pointed (7.58%) were Common Frogs. The remainder (87.9%) had no substantive differences.

Intraspecific differences
Intraspecific differences were also negligible when photographs which seemed to show large variation in pointedness (e.g. Pr1 and Pr2 in Fig. 2) were superimposed (Fig. 4 (I)). However, there were occasional measurable intraspecific differences in the distance between the anterior eye edge and the tip of the snout (e.g. P.r.1 was 1.4 times longer than P.r.2). A similarly large variation was found in the Common Frogs. This variation did not seem to have any substantive effect as the head region outlines were clearly more related to the underlying lateral head bone structure rather than relative eye position.

Differences in eye area and facial marking angles
Average eye width / eye length ratios results (Table 1B) show the brown frogs were more

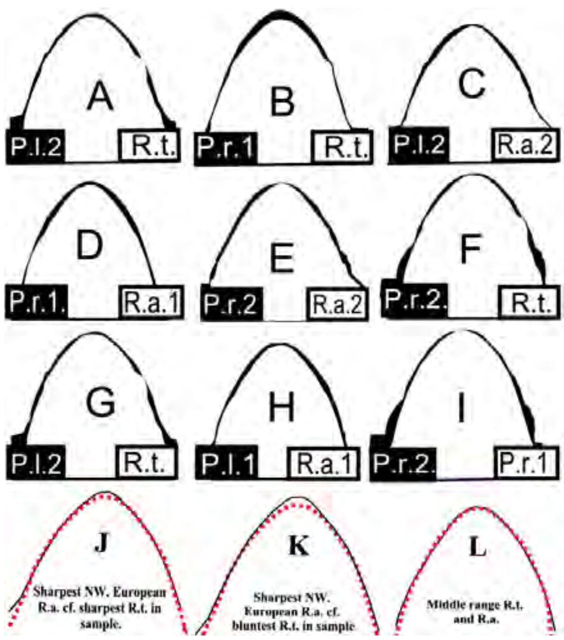

Figure 4. representative sample of the possible combinations produced from the black silhouettes (water frogs) overlain with the white silhouettes (brown frogs)

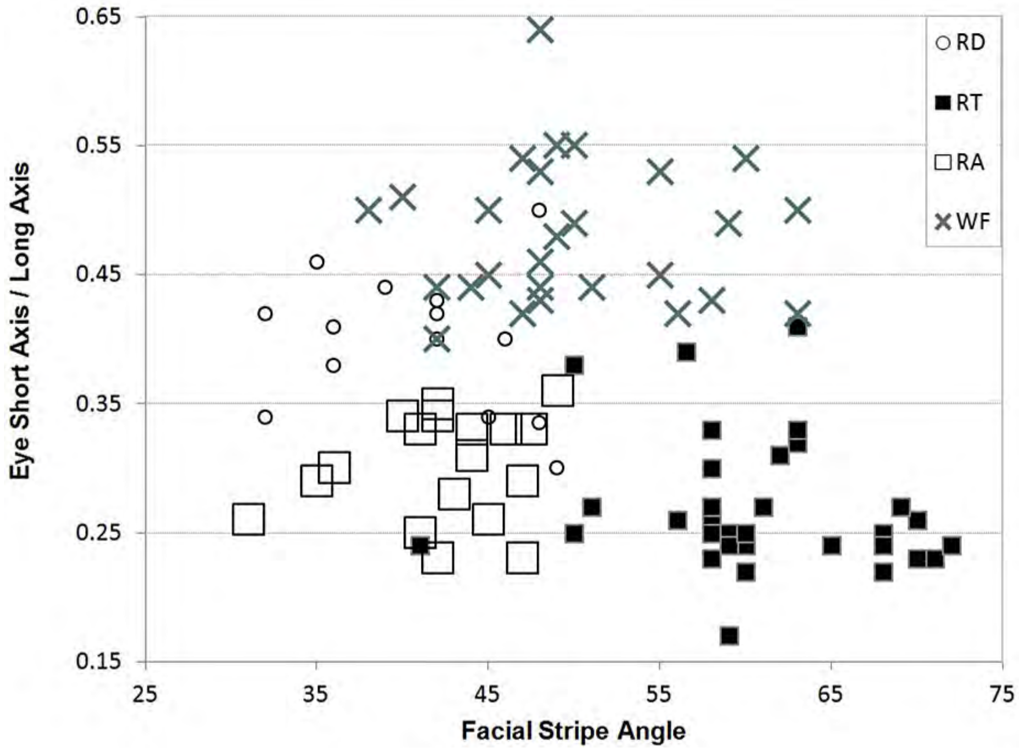

Figure 5. A scatter plot depicting eye ratios versus facial stripe angles in brown and water frogs.

laterally focused compared to the frogs; the averaged ratios were 0.33 and 0.48, respectively. Table 1B and Fig. 3 show that, viewed from above, this is particularly the case in the Moor and Common Frogs (averaging 0.3 and 0.27 respectively) which show very noticeably smaller areas of the eye than the water frogs (average 0.48). The Agile Frog average eye ratios (0.4) on the other hand were much closer to those of the water frogs, a fact emphasised in a scatter plot (eye ratios v. facial stripe angles, Fig. 5) where ca. 43% of the Agile Frog plots abut or inter-penetrate the water frog plots.

Both Moor Frogs and Agile Frogs showed an indentation in the facial lines (arrowed, Fig. 3), which was absent from water frogs and weak in the Common Frog (positions arrowed). A line drawn and aligned to the dorsal eye edge (e.g. the dotted lines in R.d. and P.l. in Fig. 3) also emphasised the indentations and straighter route taken (from the anterior of the eye to the nostril) by the snout markings in water frogs and, to a lesser degree, the Common Frog compared to the other two species.

The angles and form of the lines across the snout (Fig. 3) varied enough between the groups and species to be a potential diagnostic feature.

Statistics

Sample statistics, ANOVA and two sample t-tests results are given in Table 1.

Table 1A indicates that the Common Frog had an average angle between the markings of 60.83° (n = 33, SD 6.8), the Moor Frog angles averaged 42.6° (n = 19, SD 4.67), the Agile Frog average was 40.85° (n = 14, SD 5.93) and the water frogs averaged 49.3° (n = 27, SD 6.7).

ANOVA results (Table 1A) gave high significance values for differences between the species/groups ($p < 0.001$). ANOVA tests do not indicate where this variability lies (i.e. between which species). To remedy this, a series of two sample t-tests were carried out; the results are shown in Table 1A. This table shows that all sample comparisons yielded highly significant differences in eye stripe angle parameters between the species except in the comparison between *R. dalmatina* and *R. arvalis* ($p = 0.35$) and this remained the only case of H_O acceptance after applying Holm's sequential Bonferroni post-hoc testing.

The ratio of eye width divided by eye length (EW/EL) obtained from the eye outline tracings described earlier (Fig. 3), used one eye for each specimen. The resulting ratios (Table 1B) were:

A Facial stripe angle: Sample Statistics

Groups	RD	RT	RA	WF
N	14	33	19	27
Average	40.85°(SD, 5.93)	60.83°(SD, 6.83)	42.6°(SD, 4.67)	49.3°(SD, 6.7)

Anova	**df** (between groups)	**df** (within groups)	**f**	**p**
	3	89	50.67	< 0.001

Two-Sample t-tests: Facial stripe angles

Pair	Species	df	t	Significance
1	Water frogs v. *R. temp.*	58	-6.20	p < 0.0001
2	*R. arvalis* v. *R. temp.*	50	-10.3	p < 0.0001
3	Water frogs v. *R. arv.*	44	4.10	p < 0.001
4	*R. dal.* v. water frogs	39	-4.27	p < 0.001
5	*R. dal.* v. *R. temp.*	45	-9.51	p < 0.0001
6	*R. dal.* v. *R. arvalis*	31	-0.95	p = 0.35

B Eye Length / Eye Width: Sample Statistics

Groups	RD	RT	RA	WF
N	14	33	19	27
Average	0.4 (SD, 0.055)	0.27 (SD, 0.05)	0.3 (SD, 0.043)	0.48 (SD, 0.056)

Anova	**df** (between groups)	**df** (within groups)	**f**	**p**
	3	89	94.8	p < 0.001

2-Sample t-tests: Eye short axis length / long axis length

Pair	Species	df	t	Significance
1	Water frogs v. *R. temp.*	58	15.26	p < 0.0001
2	*R. Arvalis* v. *R. temp.*	50	2.0	p = 0.0502
3	Water frogs v. *R. arv.*	44	12.06	p < 0.0001
4	*R. Dal.* v. water frogs	39	-4.55	p < 0.0001
5	*R. Dal.* v. *R. temp.*	45	7.70	p < 0.0001
6	*R. Dal.* v. *R. Arvalis*	31	5.95	p < 0.0001

Table 1A. Facial stripe: sample statistics, ANOVA and t-test results.
Table 1B. Eye ratio (short/long axis): sample statistics, ANOVA and t-test results.
Note the highly significant p values in all tests except pair 6 in 1A and pair 2 in 1B (boxed).

all brown frogs 0.32, all water frogs 0.48, Common Frog 0.27, Moor Frog 0.3, and Agile Frog 0.4. This equates to the water frog samples here having 1.5 times as much eye area visible from above (WF/BF = 0.48/0.32) compared to brown frog samples. An ANOVA test (Table 1B) indicated very significant differences between the populations ($p = < 0.001$). Corresponding t-tests (Table 1B) indicated that all groupings had highly significant differences except in the case of *R. arvalis* and *R. temporaria* where the H_O was accepted; this remained the only case after the application of the sequential Bonferroni test. Snout angle and eye ratio results are plotted in Fig. 5.

DISCUSSION

Snout pointedness showed no value in group separation: advice that water frogs have a more pointed snout than brown frogs was inaccurate. Based on the samples available here, separation of Common and Moor Frogs using snout pointedness was also unsafe. There was a small tendency for the sharpest snouts to belong to Moor Frogs and the bluntest to Common Frogs, but these differences would be very hard to notice in the field. The majority of head outlines turned out to be indistinguishable between the species.

It has been the author's experience that younger frogs (particularly in Common and water frogs) can have relatively longer snouts (if defined as the distance from the anterior eye edge to snout tip) compared to adults, which tend with age to increasing apparent "bluntness". This phenomenon has also been reported for Common Frog (Arnold & Ovenden, 2002). Therefore, the Marsh Frog labelled P.r.2 in Fig. 2 could simply be older than the individual labelled P.r.1. If one species had a longer lifespan than another, that species may, on average, appear to have blunter head profiles. Any underlying trend for changes with age weakens the usefulness of this characteristic. The bluntening effect seen in P.r.1 and P.r.2 (Fig. 2) is predominantly associated with changes in the relative distance from the nostril to the anterior edge of the eye.

Applying a method not previously described, the facial stripes had patterns and angles which did discriminate between the two groups and between some brown frog species. The "V" formed by the two snout markings was more pointed in water frogs compared to Common Frogs. While evidence of differences in snout pointedness was not found, the Moor Frog facial markings converged at significantly smaller angles than those of the Common Frog and, possibly tellingly, the Moor Frog is also described as having a more pointed snout than the Common Frog (Matz & Weber, 1983; Laňka & Vít,1989; Fog et al., 1997, Arnold & Ovenden, 2002). References to a more pointed snout could be an illusion based on the greater pointedness of the facial markings in water or Moor Frogs compared to the Common Frog, rather than the physical structure of the snout itself.

Compared to Moor and Common Frogs, water frog eyes (viewed from above) showed relatively more area. Water and Common Frog snout stripes show greater straightness than either the Moor or Agile Frogs (Fig. 3). Hence facial detail varied widely between species and the groups.

These samples suggested that Agile Frogs also have more upwardly looking eyes than the other two brown frog species. The author has experienced, both in the wild and in outdoor enclosures, that water frogs are more likely to leap up and intercept flying insects than the Common or Moor Frogs. Having more upwardly focused eyes would benefit this mode of feeding. This suggests future research. Where the Agile Frog shares habitat with other brown frog species, it might similarly benefit from its long legs and more upwardly focussed eyes to feed in a similar way; i.e. is there a degree of habitat and trophic partitioning where the ranges overlap?

The results have suggested that differential eye ratios and facial stripe angles offer value in the identification of north-west European frog groups and species. The methods given here might also aid identification from images; it is the author's experience that agencies supplying images for publication frequently misname species. Published guidance on discrimination between these species suggest using metatarsal tubercle (MT) form (not usually visible in photographs) (e.g. Arnold & Ovenden, 2002), or temporal mask form to separate Moor and Common Frogs (Fog et al., 1997) and leg length to separate Agile from other (shorter legged) brown frog species and to help separate water frog forms (e.g. Nöllert & Nöllert, 1992; Fog et al., 1997; Arnold & Ovenden, 2002). These parameters, as with some parameters given here, overlap to some degree and some methods described in the literature (e.g. the absence of a temporal mask in water frogs) are inaccurate (Snell, 2011). Where ambiguity exists, it is desirable to have recourse to extra distinguishing methods which might provide more unequivocal separations. Detail on frogs' heads, while not easily useable in the field (but likewise leg length and MT), are potentially more useful than flawed guidance suggesting the use of snout pointedness or the presence of a temporal mask.

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