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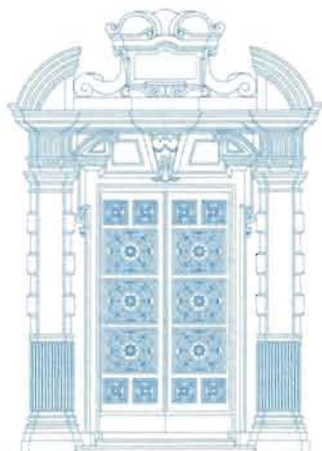

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ABSTRACT

The larvae of the recently discovered brown frog *Rana pyrenaica* differ from other European brown frog tadpoles by their dark brown to deep black dorsal colour with silvery white spots on the flanks. Tooth formula of the examined specimens was 1:3+3/1+1/3. Two distinct large and two very small lingual papillae do occur, thus representing an intermediate character state between European *Rana* species with two and with four distinct papillae.

A diagnostic difference to *Rana temporaria* tadpoles from the Pyrenees is the gap between both portions of the second upper tooth row, which is much smaller in *Rana pyrenaica*. Additionally, a multivariate morphometric comparison showed highly significant differences between tadpoles of *Rana pyrenaica* and sympatric *Rana temporaria* regarding several morphometric characters. A rather large interpopulational variability in several oral structures was found in *Rana temporaria* tadpoles from the Pyrenees.

1. INTRODUCTION

Until recently, the taxonomic status of brown frog populations from the Spanish and French Pyrenees was subject to controversial opinions. The presence or absence of *Rana dalmatina* in the Spanish part of the Basque country and in Catalonia was uncertain (Garcia-Paris, 1985), and discussions arose on the supposed - but never evidenced - presence of *Rana iberica* in the Pyrenean mountain range (see Garcia-Paris, 1985; Bea, 1989). The classification

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of Iberian grass frogs (*Rana temporaria*) as different subspecies (*R. t. canigonenis, R. t. parvipalmata*) also remained rather obscure. Long-legged pyrenean *temporaria* specimens were preliminary named "Gassers frog", pending further study (see Dubois, 1983; Nöllert & Nöllert, 1992).

Still today several open questions on Iberian brown frog systematics do remain. A general overview, however, can be summarized from recent publications:

- Several rather isolated populations of *Rana dalmatina* occur in the Spanish basque country (Arrayago & Bea, 1985). However, the presence of the species in the Pyrenees as reported in the map of Guyetant (1989) is still unclear. Records from Catalonia have been demonstrated to be erroneous (Llorente et al., 1995).

- *Rana iberica* regularly occurs in the Spanish basque country (Arrayago & Bea, 1985; Bea, 1989), but does not seem to penetrate into the montane range of the Pyrenees, neither into France.

- *Rana temporaria parvipalmata* lives in north-western Spain (Galicia and western Asturias). It is well diagnosed by allozyme genetics (Arano et al., 1993), morphology (Galan, 1989b), extended breeding period (Galan, 1989a; Vences, 1992, 1994) and possibly also bioacoustics (Vences, 1992). This taxon may even merit a rank as distinct species (Arano et al., 1993).

From central Asturias eastwards, but possibly also in mountain ranges of Galicia such as the Serra do Caurel, only grass frogs are known which clearly do not belong to *parvipalmata*. Their status (typical form or differentiated subspecies) is not sufficiently known (Arano et al., 1993; Vences, 1992).

- *Rana temporaria* shows clear interpopulational differences also in the Pyrenees. At high elevations (eg. Mont Canigou in the eastern Pyrenees, Dubois, 1983; but also in the western Pyrenees, own data) many frogs show rather short legs (tibiotarsal articulation reaching the eye). Both in Spanish populations (Vidra, Maranges following Balcells, 1956) and at lower elevations in the French western Pyrenees (eg. the low Vallée d'Aspe, own observations), specimens have much longer hindlimbs, the tibiotarsal articulation reaching between nostrils and snout tip - thus corresponding with the form named Gasser's frog by Dubois (1983). The short-legged population from Mont Canigou has been described as subspecies *canigonensis*, and this name may be applied to the short-legged forms from high altitude Pyrenees in a preliminary way according to Dubois (1983).

- Only two years ago a new species of brown frog was described from the central western Spanish Pyrenees: *Rana pyrenaica* Serra-Cobo, 1993. This species is a well differentiated, rather small, uniformly light beige coloured high mountain frog. Old records of *Rana iberica* in the Pyrenees possibly are due to confusion with this new species. So, the frog from El Pueyo de Jaca pictured by Garcia-Paris (1985) in fig. 156 as *R. iberica*-like *R. tem-*

poraria with long legs is certainly a *R. pyrenaica*.

In the present study we give a first detailed description of the larval stages of *R. pyrenaica*, a species for which we propose the English common name of Pyrenean frog. We especially focus on larval mouthpart structures and on differences to *R. temporaria* tadpoles from the Pyrenees; additionally we summarize some anecdotical observations on habitat and habits of the species, and illustrate adults and larvae in colour.

2. MATERIAL AND METHODS

Field observations were carried out by the two senior authors in the area of the Parque Nacional de Ordesa y Monte Perdido, Huesca province, Spain. Tadpoles were observed along the Rio Arazas, which constitutes the National Park border, and five tadpoles were captured at one locality in the immediate vicinity of Rio Arazas but outside the National Park (about 1500 m above sea level, near Mondicieto mountain).

Detailed measurements, mainly of oral disk structures, were taken from these five *Rana pyrenaica* tadpoles and from two *Rana temporaria* tadpoles from the Lacs d'Ayous (in the vicinity but outside the borders of the Parc National des Pyrenées Occidentales, western central Pyrenees, France) and one *R. temporaria* tadpole from the upper Vallée d'Aspe (Pool along Lescun river, near Lescun, western central Pyrenees, France). Tadpoles were fixed and preserved in the field in 70% ethanol; they were measured with a binocular with measuring device to the nearest 0.1 mm. Many more tadpoles of both species were examined in the field and in other samples (see below) and were found to agree in the main external characters.

Tadpole keratodont rows are described using the standardized formula proposed by Dubois (1995). To enable better comparison, abbreviations of measurements mainly follow Grillitsch et al. (1993). Also the terms and structure of the following description are largely based on Grillitsch et al. (1993) which is the most detailed and complete description of a European brown frog larva so far published.

The following abbreviations are used: SVL Snout-vent-length; TL total length; BH maximum body height; BW maximum body width; TaL tail length; UF height of upper (dorsal) tail fin at the point of maximum tail height; UF1 height of dorsal tail fin at the middle of the tail; UF2 height of dorsal tail fin at the beginning of the tail; LF height of lower (ventral) tail fin at the point of maximum tail height; LF1 height of ventral tail fin at the middle of the tail; LF2 height of ventral tail fin at the beginning of the tail; TM1 height of caudal musculature at the middle of the tail; TM2 height of caudal musculature at the beginning of the tail; HT maximum height of tail (including dorsal and ventral tail fin); TW1 maximum tail width at the middle of the tail; TW2 maximum tail width at the beginning of the tail; ID interocular distance; SS distance tip of snout-opening of spiracle; VS distance vent-opening of spiracle; OD maximum width of oral disk; HBW approximate width of horny beak; NE distance between nostril and eye; TsE distance between eye and tip of snout; NN distance between nostrils; ED eye diameter; SD mean diameter of white lateral spots; MP number of marginal papillae; IMP number of inframarginal papilla.

Anterior (upper) tooth rows are named UTR, posterior (lower) tooth rows LTR. They are numbered from the margin towards the center of the oral disk.

For general morphometric comparisons, additional 49 *R. pyrenaica* tadpoles from Valle de Bujaruelo (Huesca province, Spain; locality 1) and 45 *R. temporaria* tadpoles from several localities of the eastern Pyrenees were studied by the three junior authors. These localities were (locality numbers as used in fig. 5): Liat (2), Montgarri (3) and Artiga de Lin (4) -Lleida province-, Meranges (5), Ogassa (6), Ull de Ter (7) and Espinavell (8) -Girona province-, Spain). The Gosner stage of each individual was recorded. The following morphometrical variables were measured (with a digital caliper to the nearest 0.1 mm): SVL, TaL, HT, BH, BW, OD, NN, ID, NE, TsE. In order to compare the overall morphometry of both species, a Multivariate Analysis of the Covariance (MANCOVA) was performed keeping SVL as covariate to standardize the restant variables with regard to body size. Since variation throughout larval development could be expected, the sample was divided into two homogeneous groups: individuals belonging to Gosner stages lower (17 *R. pyrenaica*, 33 *R. temporaria*) and higher (32 *R. pyrenaica*, 10 *R. temporaria*) than 30. This subdivision was applied due to the distribution of stage classes in the studied sample (most specimens either in stage < 25 or > 30). After finding significant results, Scheffé's *post hoc* comparisons between both species were carried out for each group and variable.

Furthermore, individuals were plotted in two dimensions using the Principal Component Analysis (PCA) for reducing dimensionality. The factorial axes were conserved unrotated.

3. RESULTS

3.1. Adult general appearance and habitat of *Rana pyrenaica*

In general *R. pyrenaica* (pl. I.3 and I.4) is a relatively small-sized *Rana* species, phenetically belonging to the palearctic brown frogs (subgenus *Rana*; Dubois, 1992), with SVL ranges of 33-46 mm in males and 36-51 mm in females (Serra-Cobo, 1993). Dorsal colour typically is uniform and varies from beige to creme brown. Typical V-shaped pattern can be found on the dorsum in some specimens, otherwise no distinct pattern is present. Ventrally the colour is much lighter and goes into white. Nuptial pads of the males are coloured greyish or brownish. In these traits our observations are much in accordance with the findings of Serra-Cobo (1993).

In open pasture landscape of the Ordesa-valley (pl. I.1) we observed adult frogs during the day in slow running streams, sitting near or in the water. When disturbed they immediately tried to escape by hiding under stones and rocks below water surface. Vertical height was about 1800 m above sea level.

Larvae and about a dozen of recently metamorphosed juveniles were found down the valley at 1600 m (pl. I.2), in a small, slow moving branch of Rio Arazas, which formed numerous large pools with nearly stagnant water. The newt *Euproctus asper* was very common in these pools (1-2 specimens captured per meter of examined brook, without intensive searching). From this observation it can be speculated that *Euproctus* may constitute an important predator of *R. pyrenaica* larvae, although direct observations are lacking.

3.2. The tadpole of *Rana pyrenaica*

By first appearance most tadpoles of *Rana pyrenaica* are completely different from all other European brown frog tadpoles. This is mainly caused by the striking black dorsal colour, which makes many larvae closely resembling a *Bufo* tadpole by first sight. This colour pattern is the most distinctive character which generally alone allows an immediate field identification. The following description refers to larvae in Gosner stages 33-38. The larvae in earlier stages (25-29) differ in being darker and showing brighter and smaller spots on tail and body, which are more separated from each other than in the later stages. Some additional measurements of the specimens used for detailed mouthpart descriptions can be found in tab. 1 and tab. 2. Specimens, in the table as well as in the following text, are referred to as RP1 to RP5.

The whole dorsal and lateral surface of the body is black (pl. 1.6) to dark brown. The dorsum is usually black and gets clearer towards the belly due to the presence of spots which are silvery, white-yellowish or even golden and always with irregular borders. The same spots are also present on dorsal and ventral caudal fins, and caudal musculature. Their diameter is 0.25 to 0.5 mm, and on the body their size increases gradually from dorsum to belly, as does the intensity of the spotting. The belly is greyish transparent, except for the dense speckling with the white spots.

The tail, including the caudal fin, is dark, sometimes black, and shows smaller spots than the rest of the body. When observed through a binocular microscope, the background dark colour of the tail is composed of independent melanocytes whose pigment is arranged in a very ramified shape.

The tail makes up for 54-66% of the total length. The tail is high, with a rounded end. At midlength of the tail, the caudal musculature accounts for

	RP1	RP2	RP3	RP4	RP5	RT1	RT2
Gosner	33	36	38	38	37	36	36
SVL	12.0	12.5	14.0	14.4	13.3	11.4	10.8
TaL	17.2	22.8	20.4	22.6	22.2	16.2	20.4
UF	1.4	1.3	2.1	2.9	1.5	2.1	-
UF1	1.2	1.4	1.9	2.5	1.9	-	-
UF2	0.7	0.7	0.7	0.7	0.7	-	-
LF	0.8	0.8	1.0	1.0	1.2	1.8	-
LF1	0.7	0.7	0.9	1.0	1.2	-	-
LF2	0.1	0.1	0.2	0.1	0.2	-	-
TM1	1.9	2.7	2.6	2.5	2.1	2.2	2.7
TM2	1.9	2.6	2.5	2.6	2.5	-	-
HT	4.1	4.9	5.5	5.5	5.5	5.7	5.0
TW1	0.9	1.2	1.1	1.4	1.5	-	-
TW2	1.9	2.7	2.6	2.8	2.5	-	-
BW	7.5	7.8	8.1	8.6	7.8	6.5	-
BH	5.8	6.1	6.4	6.9	6.1	4.9	-
ID	3.2	3.7	4.3	4.4	4.8	3.8	-
SS	7.2	7.3	6.7	6.6	6.6	6.8	-
VS	6.4	6.7	7.3	7.2	7.8	4.6	-
OD	3.1	2.9	2.6	2.5	2.5	2.4	2.0
HBW	0.8	1.0	1.1	1.0	1.0	-	-
NE	1.8	2.3	2.4	2.4	2.1	-	-
NN	2.3	2.4	2.1	2.1	2.1	2.1	-
ED	1.3	1.5	1.5	1.7	1.6	1.3	-

Table 1. Morphometric measurements (in mm) of five *Rana pyrenaica* (RP) tadpoles and two *Rana temporaria* (RT) tadpoles which were used for detailed description and measurements of mouth-part structure. RP specimens from near Rio Arazas, in the vicinity of the Parque Nacional de Ordesa y Monte Perdido, Huesca province, Spain, RT specimens from Lacs d'Ayous, central western Pyrenees, France. For abbreviations see the Material and Methods chapter.

	RP1	RP2	RP3	RP4	RP5	RT1	RT2
SS/VS	1.13	1.08	0.92	0.92	0.85	1.48	-
HT/TaL	0.24	0.22	0.27	0.24	0.25	0.35	0.25
UF/HT	0.34	0.27	0.38	0.53	0.27	0.37	-
NN/ID	0.72	0.65	0.49	0.48	0.44	0.55	-
OD/ID	0.97	0.78	0.61	0.57	0.52	0.63	-
TM1/HT	0.46	0.55	0.47	0.46	0.38	0.39	0.54
SVL/TL	0.41	0.35	0.41	0.39	0.38	0.41	0.35

Table 2. Morphometric ratios of *Rana pyrenaica* and *Rana temporaria* tadpoles. Same specimens and abbreviations as in table 1.

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Fig. 1. Drawing of the o
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about 40-50% of total tail height.

The spiraculum is sinistral, about equidistant between the snout and the vent and seems to be oriented to the rear part in our specimens. The central anus oriented to the right side.

The eyes are close together and dorsally oriented. The iris has golden pigment. A poorly defined yellow-golden superciliary stripe can be found in some individuals.

The general shape of the tadpole is robust and dorsally pyriform. The snout is sharp but apically truncated. The oral disk (fig. 1, fig. 3) is in ventral subterminal position. Marginal peribuccal papillae (MP) are restricted to the lateral corners and the posterior margin of the disk, reaching anteriorly more or less the lateral beginnings of the outermost upper tooth row (UTR1). In RP1 (stage 33) they were mainly arranged in a single row, with a density of about 11 per millimetre. Solitary inframarginal papillae occurred irregularly along the posterior margin of the disk but were mainly clustered in the disk corners. No papillate ridges descending from the disk corners to the beak could be seen as distinctly in our specimens as was described and shown for

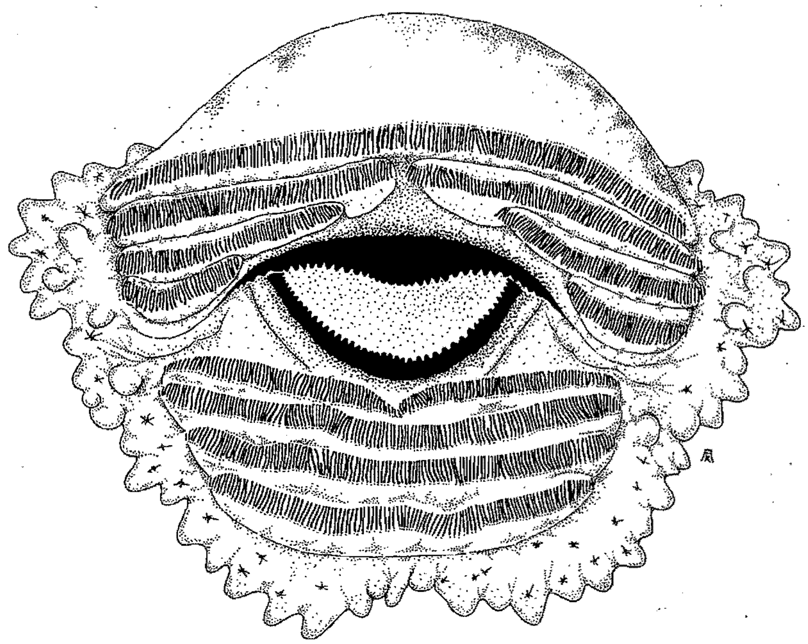


Fig. 1. Drawing of the oral disk and associated structures of a *Rana pyrenaica* tadpole from near Rio Arazas, in the vicinity of the Parque Nacional de Ordesa y Monte Perdido, Huesca province, Spain.

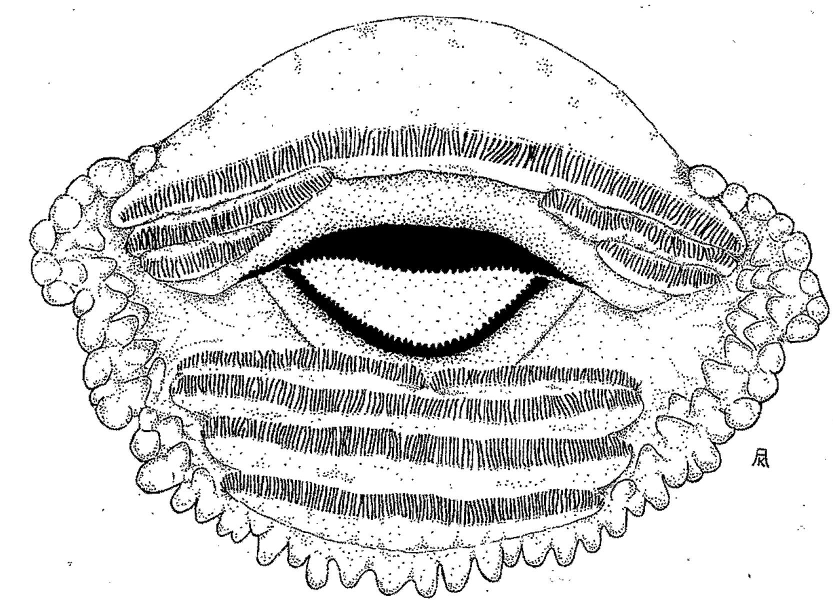


Fig. 2. Drawing of the oral disk and associated structures of a *Rana temporaria* tadpole from the Lacs d'Ayous, central western Pyrenees, France

Fig. 3. Oral disk of a *Rana pyrenaica* tadpole from near Río Arazas, in the vicinity of the Parque Nacional de Ordesa y Monte Perdido, Huesca province, Spain.

Rana graeca and *Rana italica* by Grillitsch et al. (1993). However, as can be recognized in fig. 3, scarsely developed ridges in the disk corners did in fact occur in our *R. pyrenaica* tadpole specimens. In RP1 (stage 33) and RP3 (stage 38), number of MP was 44 and 40, number of IMP 20 and 8, respectively.

In all examined tadpoles the number of keratodont rows (tooth rows) was eight, four on the anterior lip and four on the posterior lip. Detailed keratodont formula was 1:3+3/1+1:3. Keratodonts were disposed as single series; their density in RP1 (stage 33) was 50 per millimetre in the second tooth row of the upper lip (UTR2).

Length of the four continuous rows was 2.3 mm (UTR1) in two tadpoles;

GOSNER STAGE <30

Variable	*R. pyrenaica* (n=17) mean	std err	min.	max.	*R.temporaria* (n= 33) mean	std err	min.	max.
SVL	8.41	0.23	7.0	10.0	7.42	0.34	4.0	11.0
TaL	14.35	0.41	12.0	19.0	11.94	0.41	8.0	17.0
HT	4.31	0.15	3.0	5.0	3.17	0.14	1.5	4.5
BH	4.02	0.20	3.0	5.5	3.28	0.15	1.2	5.0
BW	5.53	0.24	3.5	7.5	4.61	0.17	2.5	7.0
OD	1.81	0.04	1.5	2.1	1.97	0.10	0.9	3.0
NN	0.96	0.03	0.7	1.2	1.13	0.06	0.7	2.4
ID	1.76	0.06	1.3	2.2	1.76	0.10	0.9	2.8
EN	0.95	0.01	0.9	1.0	0.96	0.06	0.4	1.7
TsE	1.92	0.05	1.6	2.3	2.02	0.10	0.9	3.0

GOSNER STAGE >30

Variable	*R. pyrenaica* (n=32) mean	std err	min.	max.	*R.temporaria* (n=10) mean	std err	min.	max.
SVL	10.94	0.14	9.5	13.0	12.00	1.10	6.0	18.0
TaL	17.25	0.27	13.5	21.0	20.60	1.84	11.0	28.0
HT	4.48	0.12	3.0	5.5	5.00	0.49	3.0	8.0
BH	5.41	0.21	4.0	10.0	5.35	0.55	3.0	8.0
BW	7.38	0.11	6.5	9.0	8.00	0.76	4.0	12.0
OD	2.44	0.04	2.0	2.8	2.57	0.15	1.7	3.2
NN	1.36	0.05	1.0	2.0	1.82	0.18	0.9	2.5
ID	2.28	0.05	1.8	2.9	3.10	0.33	1.6	5.0
EN	1.41	0.05	0.9	2.0	1.42	0.13	0.9	2.0
TsE	2.46	0.05	1.6	3.0	2.55	0.23	1.7	4.0

Table 3. Descriptive statistics of *R. pyrenaica* tadpoles (from Valle de Bujaruelo, Huesca province, Spain), and of *R. temporaria* (from several localities in Lleida and Girona provinces, eastern Pyrenees, Spain; see Material and Methods chapter for exact localities). Early and later larval stages are considered separately. All measurements in mm.

1.7 mm (LTR1), 1.9 mm (LTR2), 2.0 mm (LTR3) in RP1 (stage 33). Length of the interrupted upper tooth rows (UTR2, UTR3, UTR4) decreased in centripetal direction. In the same direction, the length of the gaps between both portions clearly increased, although the exact length of the gaps was difficult to measure. Length of one portion of the rows was 1.2 and 1.3 mm (UTR2), 0.8 and 0.9 mm (UTR3), 0.5 and 0.4 mm (UTR4); approximate length of the gap was 0.1 mm (UTR2), 0.8 and 0.9 mm (UTR3), 1.1 and 1.5 mm (UTR4) in RP1 (stage 33) and RP2 (stage 36), respectively. The gap in UTR2 was rather small, the ratio between one portion of the row and the gap being 10.4 and 16.3 in RP1 and RP2.

The only interrupted lower tooth row (LTR4) showed a small central gap which could not always be immediately recognized. Length of the gap was < 0.1 mm, length of each row portion about 1.0 mm in RP1 (stage 33).

The central horny beak was robust and dark pigmented; both jaw sheaths had serrations at their cutting edge.

No detailed measurements and counts were performed on the structures of the buccopharyngeal cavity (buccal roof and buccal floor) except for the number and size of the lingual papillae in RP3 (stage 38), RP4 (stage 38) and RP5 (stage 37). We detected four lingual papillae. Two distinct, rather long and slim cylindric papillae rised centrally in the posterior part of the tongue anlage. Two additional papillae were very short and could only be

Fig. 4. Oral disk of a *Rana temporaria* tadpole from the Lacs d'Ayous, central western Pyrenees, France.

detected after careful examination. They were situated somewhat more laterally than the large central papillae.

A single tadpole from the same locality as RP1 to RP5 was reared until metamorphosis. The metamorphosing froglet measured 11.5 mm SVL. During metamorphosis the body became light brownish, while the tail or tail stump was still black. Colouration change first occurred on the back, with a distinct dorsilateral border between brown back and still blackish flanks (pl. I.5). The silvery white spots were still distinct on the flanks, giving the specimens a vivid golden appearance. Metamorphosed young, as observed in the field, are already completely brown and colouration soon changes to light beige.

3.3. Morphological comparison between tadpoles of *Rana pyrenaica* and *Rana temporaria*

Tab. 3. shows the morphometric values obtained for those specimens which were considered for the morphometric analysis. Although the largest tadpoles belonged to *R. temporaria*, no differences in the body size (SVL) were detected between both species neither in the early stages nor in the advanced ones. However, significant overall differences were found in the shape of the two species for the stages lower than 30 (MANCOVA, Wilks' Lambda = 0.3077; Rao's R = 9.7479; 9, 39 d.f., p < 10-6). The *post hoc* tests revealed significant differences, independent from body size, for the variables TaL, HT, BH, BW and NN (see tab. 4).

Differences were also found for the stages higher than 30 (MANCOVA, Wilks' Lambda = 0.3454; Rao's R = 6.5272; 9, 31 d.f., p = 3.6*10-5). After the *post hoc* tests, the variables with significant differences in this case were

Variable	Gosner < 30	Gosner > 30
TaL	6*10-6	5.3*10-5
HT	0	0.0017
BH	5.6*10-5	N.S.
BW	1.9*10-5	2.1*10-4
OD	N.S.	N.S.
NN	0.012	1.8*10-5
ID	N.S.	0
EN	N.S.	N.S.
TsE	N.S.	N.S.

Table 4. Results (p values) of the *post hoc* Scheffé's test between *R. pyrenaica* and *R. temporaria* considering the early and later larval stages separately. N.S. not significant. Refers to data shown in table 3.

TaL, HT, BW, NN and ID (tab. 4). Mean values, relative to SVL, of these variables are given in table 6.

In fig. 5, the individuals of both species are projected over the first two factorial axes. Tab. 5 shows the correlation coefficients between each variable and these two axes for both analyses as well as the explained variance in every case.

Beside these morphometric differences, the following general morphological features were found to be generally diagnostic between tadpoles of the two species: 1) In *R. temporaria* the snout is more rounded, not truncated. 2) *R. temporaria* shows less and smaller silvery spots than *R. pyrenaica*. 3) Under the binocular microscope, the melanocytes in the tail of *R. temporaria* are connected (not independent) forming a reticula.

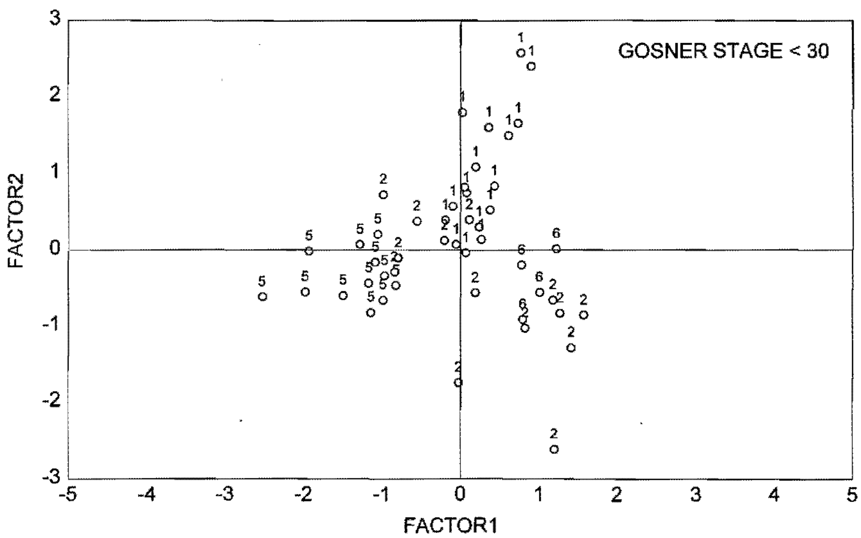
3.4. Preliminary data on oral structures of *Rana temporaria* tadpoles from the Pyrenees

The detailed study of oral structures of *R. temporaria* tadpoles from two localities in the central western Pyrenees showed rather large differences between the two samples.

Tadpoles from the Lacs d'Ayous (Gosner stage 36), which occurred in shallow water of sun-exposed ponds, were rather small. Their tooth formula was 1:2+2/1+1:3. The continuous tooth rows of one specimen (RT1 in tab. 1; stage 36) measured 1.9 mm (UTR1), 0.8 mm (LTR1), 1.5 mm (LTR2), 1.1 mm (LTR3). One row portion of the interrupted tooth rows measured 0.6

	Gosner stage <30		Gosner stage >30	
Variable	**Factor 1**	**Factor 2**	**Factor 1**	**Factor 2**
SVL	0.94*	0.07	0.95*	0.07
TaL	0.76*	0.42	0.81*	0.36
HT	0.74*	0.49	0.85*	0.11
BH	0.79*	0.31	0.70*	-0.45
BW	0.76*	0.51	0.92*	0.03
OD	0.84*	-0.38	0.74*	-0.01
NN	0.50	-0.59	0.80*	0.19
ID	0.87*	-0.30	0.91*	0.28
EN	0.86*	-0.29	0.73*	-0.51
TsE	0.88*	-0.29	0.86*	-0.24
Explained var.	64.57	15.36	69.57	7.81
Cum.Expl.var.	64.57	79.93	69.57	77.38

Table 5. Correlation coefficients between the biometrical variables and the first two axes of the principal component analysis considering the early and later larval stages separately. Refers to data shown in table 3. * significant values ($p < 0.01$).



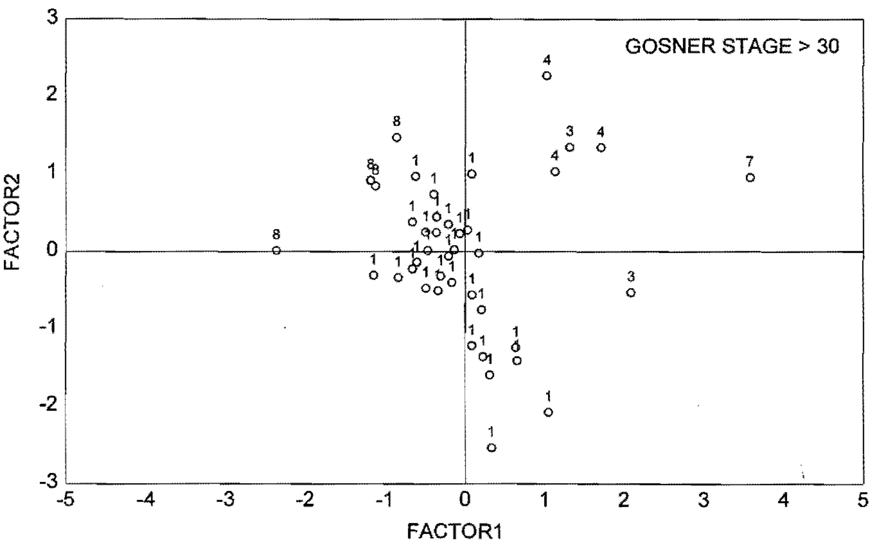


Fig. 5. Plot of *Rana pyrenaica* (1) and *R. temporaria* (2-8) individuals on the first two axes of the principal component analysis considering the early and later larval stages separately. Refers to data listed in table 3. For explanation of *R. temporaria* locality numbers see Materials and Methods chapter.

mm (UTR2), 0.2 mm (UTR3). Gaps between interrupted upper tooth rows measured 0.5 mm (UTR2), 1.3 mm (UTR3). The gap between both portions of UTR2 was rather large, the ratio between the gap and one portion of UTR2 being 1 and 1.25 in RT1 and RT2, respectively. Keratodont density was 60 per millimetre in UTR2 of RT1.

The number of marginal papillae was 34, the number of inframarginal papillae was 16 in RT1. Inframarginal papillae were disposed as parallel row behind the marginal papillae from the disk corners to the outer fourth of the posterior disk margin on both sides. Four distinct and large lingual papillae were recognizable. Measurements of the examined specimens can be found in tab. 1. The oral disk is shown in figs. 2 and 4.

One tadpole from the upper Vallée d'Aspe differed from the description given above. SVL was 10.1 mm (tail injured), OD 2.9 mm, tooth formula 1:3+3/1+1:3. Unfortunately the stage of this specimen could not be ascertained due to injuries of posterior body, but it was certainly > 30. The gap between both portions of UTR2 was rather large, the ratio between the gap and one portion of UTR2 being 2.1. Number of MP was 46, of IMP 34. This tadpole was remarkable because despite the rather good conservation of the mouthparts we were unable to detect a second pair of lingual papillae. Only one pair of large papillae could distinctly be seen on the central part of the tongue anlage.

4. DISCUSSION

4.1. High mountain adaptations in anuran tadpoles and conservation aspects

At present knowledge, *Rana pyrenaica* is the only European anuran species which is restricted to high mountain habitats. It is known from altitudes between 1200 and 1700 m according to Serra-Cobo (1993); after own data it occurs up to about 1800 m along the Rio Arazas in the Parque Nacional de Ordesa. Most of its tadpoles differ from those of all other European *Rana* primarily by the striking blackish colouration. This dark colour is possibly to be regarded as an adaptation to the cold climate and/or high ultraviolet radiation at high elevations, what would be a new hypothetic ecological correlate of tadpole colour in addition to those summarized by Altig & Channing (1993). Several examples of black colouration of high mountain amphibians and reptiles are known. Beside melanistic morphs of *Vipera berus*, in the Alps, the most obvious examples are *Salamandra atra* and *S. lanzai*. Also high mountain populations of *Rana temporaria* have an increased number of black markings and spots on the back (Nöllert & Nöllert, 1992) as the North American mountain frog *Rana cascadae* (Behler & King, 1979).

Other examples of black high mountain tadpoles can be found in the Neotropics (genus *Atelopus*; see Lötters, 1996, for references) and in Madagascar. Larvae of the related Malagasy species *Boophis williamsi* and *Boophis laurenti* from elevations of 1500-2650 in the Ankaratra and Andringitra mountain ranges of central Madagascar are predominantly blackish on the back (Blommers-Schlösser, 1979; Glaw & Vences, 1994). However, other specializations to rheophilic life shown by larvae of these species, such as a broad mouth and an increased number of tooth rows, are not shared with *R. pyrenaica*. Although it has been stated (Serra-Cobo, 1993) that the Pyrenean frog only inhabits mountain streams, we observed the species in slow-moving side branches of brooks, or in temporal ponds of quiet water near brooks.

During the last years, the phenomenon of a possible global amphibian decline has been increasingly discussed. Independently from whether this phenomenon is really a worldwide tendency or not, it remains true that in several high mountain species drastic population declines have been demonstrated. In the nearctic ranids *Rana muscosa* and *Rana cascadae* (both species restricted to mountain ranges: 365 to 2300 m, and 900 to 2700 m above sea level, respectively), heavy declines, even in national parks, occurred (Blaustein & Wake, 1995; Wake, 1991). Long term studies on populations of *Rana cascadae* gave strong evidence that high amounts of ultraviolet radiation due to the dwindling ozone shield caused a greater mortality in eggs and embryos (see Blaustein & Wake, 1995, for a summary). Since ultraviolet radiation increases with altitude, a similar danger exists potentially for each high mountain amphibian. Some species may be more affected than others, but it can not be excluded that the phenomenon will also threaten the survival of *R. pyrenaica*.

A careful long term observation of the status of the Pyrenean frog populations seems therefore a quite important measure. As far as known, the species occurs widely in the Parque Nacional de Ordesa y Monte Perdido, and therefore seems safe from habitat destruction. Nevertheless, its limited distribution and possible threats by ultraviolet radiation may well predispose *R. pyrenaica* as one of the European amphibian species on which highest conservation priority must be focused in the future.

4.2. Comparison with tadpoles of *Rana temporaria* and other European brown frogs

Our morphometric analysis demonstrates that conspicuous shape differences occur between *R. pyrenaica* and *R. temporaria* tadpoles. *R. pyrenaica* larvae in earlier stages show shorter and higher tails, more robust bodies and lower distances between nostrils than those of *R. temporaria*. Nevertheless, some of these traits change drastically during the larval development. So, the *R. pyrenaica* stages higher than 30 are conversely more short-tailed and with a similar robustness in comparison with *R. temporaria*. Only the distance between nostrils remains lower in *R. pyrenaica* than in *R. temporaria*. Moreover, in the advanced stages, the interocular distance - lower in the Pyrenean frog - is a good variable to distinguish both species.

The factorial analysis (fig. 5) illustrates these differences. Factor 1 can be considered a size dimension (see tab. 5), and factor 2 would be dependent on shape. So, for Gosner stages lower than 30, both species are perfectly separated along the shape axis. *R. temporaria* shows more size variation than *R. pyrenaica*, possibly corresponding to different life-history strategies at different localities. When considering stages higher than 30, the separation is slighter, probably because of an increment of the heterogeneity of *R. temporaria*. Nevertheless, *R. pyrenaica* remains homogeneous and clearly separated from the former.

The factorial analysis shows two clearly separated groups of specimens in the group of *temporaria* tadpoles in stages < 30, and a rather important separation between populations in the small sample of tadpoles in stages > 30 (fig. 5). However, as these separations are mainly based on size differences (specimens separated along factorial axis 1), it can be stated that the observed shape differences between *R. pyrenaica* and *R. temporaria* (see above) are consistent, and clearly not restricted to one of possibly several different morphs of pyrenean *R. temporaria* tadpoles (see also chapter 4.3.). The same interpretation is also inferred by the very high significance levels of most of the observed differences (see tab. 4). The clustering of *R. temporaria* larvae from different localities, which was necessary due to the rather small sample size, is therefore justifiable for the purpose of comparison with *Rana pyrenaica* larvae.

For field diagnosis of *R. pyrenaica* tadpoles, and discrimination from sympatric *R. temporaria* tadpoles, body shape differences as identified above can have some value, but the most obvious trait is general colouration and spot pattern. Beside this, tadpoles of *R. pyrenaica* can easily be distinguished from *R. temporaria* tadpoles by the size of the gap between both portions of UTR2. This gap is small in *R. pyrenaica*, large in *R. temporaria* (ratio tooth row portion length : gap length 10-16 versus 1-2). The same character can be used for distinction from *Rana dalmatina* tadpoles (own data of specimens from Bonn, Germany). Moreover *R. dalmatina* tadpoles, as well as *Rana iberica* tadpoles, generally lack UTR4 (Boulenger, 1891; Nöllert & Nöllert, 1992) which is present in *R. pyrenaica*. Nothing is known about the mouthpart structure of *R. temporaria parvipalmata*; descriptions (Galan, 1982) focus only on external morphometry and colour patterns.

In two characters *R. pyrenaica* tadpoles are more or less intermediate between the character states up to now found in European *Rana*.

The number of lingual papillae are considered to have diagnostic value between Central European brown and green frogs: four in the brown frogs

Rana arvalis, *Rana dalmatina*, and *Rana temporaria*; two in the green frogs of the *Rana esculenta* complex (Viertel, 1982). The brown frogs *Rana graeca* and *Rana italica* with two papillae, however, do not fit into this classification (Grillitsch et al. 1993). *R. pyrenaica* shows two large and two very small lingual papillae and therefore represents either the way towards a reduction or an amplification of papillae number. This expression of an intermediate state reduces the value of this character for establishing species groups or subgenera within *Rana* until data of more species become available.

Grillitsch et al. (1993) considered the papillate ridges in the corners of the oral disks of *Rana graeca* and *Rana italica* tadpoles as a possible support of the suctorial function of the disk and thus as an adaptation to flowing water. As stated by Grillitsch et al. (1993), such ridges had not been reported before in anuran larvae. *R. pyrenaica* larvae show weakly developed structures which can be homologous to the ridges of *R. graeca* and *R. italica*; it may be concluded that *R. pyrenaica* tadpoles, which seem to live mainly in slow moving sidearms of mountain brooks and nearby pools, are less adapted to fast flowing water than tadpoles of the two former species.

4.3. Systematics of *Rana temporaria* from the Pyrenees

The differences between *R. temporaria* populations of the Pyrenees are remarkable. In the introduction we already summarized the most important differences which were up to now detected in adult frogs. Our morphometric analysis showed that the studied sample of *temporaria* tadpoles (from several localities) contains a larger heterogeneity than the sample of *pyrenaica* tadpoles (from one locality). Although this is mainly due to size differences between populations, a smaller shape component also exists.

Especially remarkable are the differences in generally rather constant characters of tadpole oral morphology which we detected in western pyrenean specimens. So, intraspecific variation in tooth row number is known but is relatively uncommon in most taxa (Altig & Johnston, 1989). The tadpole tooth formula of *R. temporaria*, especially the development of UTR4, is known to be subject to ontogenetic changes (Grillitsch & Grillitsch, 1989). However, in the Pyrenees tooth formula differences are rather constant within and variable between populations, as we observed in a rather large number of specimens in the field. Similar differences (mainly interpopulational differences in absence or presence of UTR4) are also known for grass frog populations of the Basses Alpes in France and were used to diagnose the subspecies *R. temporaria honnorati* (see Heron-Royer, 1881; Arillo & Baletto, 1966; Sperling et al., 1996). Boulenger (who rejected a taxonomic relevance of these differences) quoted reports on the absence of UTR4 in specimens from Germany and the Alps; he also stated that "British specimens usually have only three series of upper labial teeth" (but pictured mouthparts of a specimen from near London with four upper labial tooth rows). Balcells (1956) already had noted that tooth row number in *R. temporaria* from the Pyrenees was similar to *R. t. honnorati.*

We did not attempt to correlate differences in oral structures with morphometric differences. Such an analysis lies beyond the scope of the present paper, and would have required a much larger sample of *R. temporaria* larvae; we will treat this subject in a forthcoming study. Nevertheless our data show that regarding several traits there is considerable variation in tadpole morphology of *Rana temporaria* from the Pyrenees, and we can advance two possible explanations of this phenomenon:

A) Different well differentiated parapatric and probably even sympatric and syntopic brown frog morphs could co-exist in the Pyrenees. In fact Arano et al. (1993) did not consider *R. temporaria* populations from the Pyrenees in their study of genetic variation of Iberian brown frogs; nothing is therefore known about possible genetic differentiation between these populations.

B) The tooth formulas (and morphometric traits) could depend on autecological and synecological factors such as climatic conditions, pool depth, temperature, food availability and tadpole density. Such a correlation has already been demonstrated by Hillis (1982) for the number of oral papillae. Especially an autecological correlation with the climate seems possible, since in *R. temporaria* the speed of the development from egg to metamorphosis seems to increase with lower durations of the vegetation period at the spawning sites. In Switzerland development takes only two months in samples from 2000 m altitude, but 5 months in samples from 400-600 m altitude (Aebli, 1966). This could result in a heterochrony of the development of different organ systems in high mountain populations, the tadpoles metamorphosing with a paedomorphic feature such as a lower number of tooth rows. However, Balcells (1956) found no differences in developmental rate between pyrenean and prepyrenean *R. temporaria* populations.

Conclusions from our data concerning interpopulational differences in the number of lingual papillae must be drawn carefully, since only a small number of specimens was studied and only one aberrant specimen recorded. Central European *R. temporaria* tadpoles have four distinct lingual papillae (Viertel, 1982 and own data from populations around Bonn), as we found them in the tadpoles from the Lacs d'Ayous. If, however, further studies would corroborate a constant number of two lingual papillae in tadpoles from the upper Vallée d'Aspe, this difference in a character which was used for intrageneric species grouping (Viertel, 1982; Grillitsch et al., 1993) could even be one argument to support a distinction at the species level between two *R. temporaria* morphs from the Pyrenees.

The discovery of such a distinct species as *R. pyrenaica* in 1993 and the progress in the knowledge of pyrenean mountain lizards (one endemic subspecies *Lacerta monticola bonnali* at the end of the eighties [Barbadillo, 1987]; two endemic species with one subspecies, *Lacerta b. bonnali, L. b. aranica, Lacerta aurelioi* today [Arribas, 1993a; 1993b; 1994]) shows that surprises and new discoveries can still be expected from the herpetofauna of this region.

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RIASSUNTO

È stato condotto uno studio al fine di caratterizzare le larve di *Rana pyrenaica*, una specie di "rana rossa" di recente descrizione. In base all'analisi dell'aspetto esteriore esse si differenziano dalle larve di altre "rane rosse" europee principalmente per la colorazione del dorso, variabile dal marrone scuro al nero, con punteggiatura bianco-argentata sui fianchi. La formula dentaria degli esemplari esaminati è 1:3+3/1+1/3; sono inoltre presenti due papille grandi e distinte e due papille piccole, un carattere pertanto intermedio fra quello delle specie europee di *Rana* con due e con quattro papille. Una differenza diagnostica rispetto ai girini delle popolazioni pirenaiche di *Rana temporaria* è la presenza di uno iato fra ambedue le porzioni della seconda fila superiore di cheratodonti, significativamente più piccolo in *Rana pyrenaica*. Un'analisi multivariata ha anche evidenziato differenze significative in diversi caratteri morfometrici dei girini di *Rana pyrenaica* rispetto a quelli di popolazioni simpatriche di *Rana temporaria*. È stata inoltre riscontrata una variabilità interpopolazionale relativamente elevata in diverse strutture orali nei girini di popolazioni di *Rana temporaria* provenienti dai Pirenei.

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NOTE ADDED IN PROOF

While the present paper was in press, we became aware of a recent study in which morphological variation of *Rana temporaria* in the Spanish Pyrenees (Aragon province) is discussed [Palanca Soler A., Rodríguez Vieites D. & Suárez Martínez M., 1995. Contribución al estudio anatómico del género *Rana* L., 1758 en el Alto Aragón.- Lucas Mallada, Huesca, 7: 227-247]. The authors mention the identification of two distinct, syntopic brown frog morphs which clearly differ from *Rana dalmatina, R. pyrenaica* and *R. iberica,* respectively. They attribute one of these morphs to *Rana temporaria* and name the second one *Rana aragonensis.* This observation corroborates our observation of interpopulational variability of Pyrenean *R. temporaria;* it can thus not be excluded that Pyrenean populations hitherto attributed to *R. temporaria* may refer to at least two different species. Unfortunately, the authors use the name *R. aragonensis*, which was originally meant as preliminary name for a morphotype, together with a rather detailed diagnosis, so that the name must be considered as valid in the sense of the International Code of Zoological Nomenclature. A publication in preparation by M. Vences, A. Palanca, D. Rodriguez Vieites & S. Nieto Roman will therefore stabilize the name by designation of a lectotype. Karyological and morphological studies, as well as molecular investigations on mitochondrial DNA sequences, are also in progress to ascertain the status of *R. aragonensis.*

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PLATE

1. Habitat of *Rana pyrenaica* in the Parque Nacional de Ordesa y Monte Perdido, Huesca province, Spain, along Rio Arazas. Altitude ca. 1800 m.

2. Breeding habitat of *Rana pyrenaica* in the Parque Nacional de Ordesa y Monte Perdido, Huesca province, Spain. Altitude ca. 1600 m. Tadpoles were common in this sidearm of the Arazas river.

3. Adult male of *Rana pyrenaica* from locality figured in I.1.

4. Adult female of *Rana pyrenaica* from locality figured in I.1. Ventral view

5. Metamorphosing juvenile of *Rana pyrenaica* from near Rio Arazas, in the vicinity of the Parque Nacional de Ordesa y Monte Perdido, Huesca province, Spain.

6. Tadpole of *Rana pyrenaica* from near Rio Arazas, in the vicinity of the Parque Nacional de Ordesa y Monte Perdido, Huesca province, Spain.