

Phenotypic plasticity of anuran larvae: environmental variables influence body shape and oral morphology in *Rana temporaria* tadpoles

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Abstract

Environmental variables shaped the morphology of tadpoles of the common frog, *Rana temporaria*, in various ways at the Pyrenean locality Circo de Piedrafita. Examining only specimens in similar developmental stages, those from small ponds (with higher temperature and higher tadpole density) had lower growth rates, lower relative tail height, lower relative body width and fewer labial keratodonts and keratodont rows. The variation in keratodonts may have been caused by heterochrony related to the slower growth rate. The number of lingual papillae also differed between ponds but was not related to pond size. Higher predator densities caused a higher percentage of damaged tails and a lower relative tail length in specimens with apparently intact tails, probably as a result of incomplete regeneration after mutilations earlier in development.

Key words: Amphibia, Ranidae, *Rana temporaria*, tadpoles, phenotypic plasticity, pond size, predator density, oral structures

INTRODUCTION

Time to metamorphosis and rate of development for anuran tadpoles is influenced by the temperature (Harkey & Semlitsch, 1988), size (Pearman, 1993) and rate of desiccation (Newman, 1989) of their habitat (Denver, 1997), plus density (e.g., Gromko *et al.*, 1973; Miranda & Pisanó, 1993), pathogens (Beebee, 1995; Petranka, 1995) and diet (Kupferberg, 1997). Differences in developmental rate are also caused by genetic factors in at least some species (Berven, 1987; Blouin, 1992). There is less information published on the morphological plasticity of amphibian larvae with the possible exception of size differences. Crump (1989) and Inger (1992) reported morphological differences seen in conspecific feeding and non-feeding tadpoles, while geographic variation in mouthpart morphology of *Scaphiopus* tadpoles is known from North America (Potthoff & Lynch, 1986). In salamanders (Pfennig & Collins, 1993; Nishihara, 1996) and anurans (Pfennig, 1992), cannibalistic morphs, which differ drastically from non-cannibalistic morphs in head morphology, can occur under certain environmental conditions. Predator-induced morphological changes are also

known to occur in *Hyla chrysoscelis* tadpoles (McCollum & Leimberger, 1997). A number of morphological variables and colour pattern differences between brook-dwelling and pond-dwelling larvae of *Rana chiricahuensis* are known to exist (Jennings & Scott, 1993), while the number of oral papillae may differ depending on environmental variables in *Rana berlandieri* and *R. sphenoccephala* (Hillis, 1982). Although a low percentage of specimens with aberrant oral morphology are generally present in tadpole populations (e.g. Bresler & Bragg, 1954), a high proportion of intra- or interpopulational variation of such features is mostly considered as an exception. Field guides usually use tadpole morphology as species-specific, i.e. genetically determined.

Vences, Kupfer *et al.* (1998) noted important interpopulational variation in the morphology of tadpoles of the common frog *Rana temporaria*. The systematics of Pyrenean brown frogs were not well assessed until recently (see Serra-Cobo, 1993; Vences, Kupfer *et al.*, 1998), and differences in the number of labial keratodont rows and lingual papillae have been considered as indications of taxonomic distinctness of the respective populations (Vences, Kupfer *et al.*, 1998). A second possible explanation given by the same authors was a possible heterochronic reduction of tooth rows caused by increased developmental rates.

During recent fieldwork in the Spanish central Pyrenees, large morphological variations of body shape and

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oral morphology were observed among tadpoles inhabiting neighbouring ponds. To analyse the interaction of different ecological influences producing this variation, assessments were made of the biotic and abiotic characteristics of 14 ponds and the morphology of the tadpoles inhabiting them.

MATERIALS AND METHODS

Abiotic pond characteristics

Fieldwork was carried out in the Circo de Piedrafita area, central Pyrenees, Aragón, Spain, in July 1999. This plain of 2–3 km² at c. 2100–2250 m elevation includes a number of large glacial ponds, smaller ponds and stagnant brook tributaries, which are used for reproduction by a large *Rana temporaria* metapopulation. The 14 ponds surveyed, labelled A–N, were located between 42°48.35'N–42°49.04'N and 0°15.59'W–0°17.77'W.

Ponds were classified (Table 1) as large (A–D) with a water depth > 50 cm, and small (E–N). Several small ponds were not permanent; pond N dried out at the beginning of July, killing all tadpoles (only a limited number of morphological variables could therefore be measured in the specimens from this pond). In 1998, the year before we carried out the systematic fieldwork, pond H also dried out before the tadpoles metamorphosed. At the end of the study period, ponds F, J and L were also beginning to dry up. Although most ponds were stagnant, pond M was connected to a small permanent brook, and pond K was a slow-running part of that same brook. All ponds were totally sun-exposed. The bottom of the ponds were stone or gravel, often largely covered by mud. Measurements were taken of minimum and maximum pond diameter, circumference and maximum depth, and an estimate of pond surface was calculated. As most ponds had a more or less regular depth over most of their surface, water volume was roughly estimated using pond surface and maximum depth as parameters.

Diurnal water temperature (measured in all ponds between 16:00 and 17:00 h on 16 July 1999 to exclude diel or temporal variations) was highest in the shallow ponds (23.5–30.5 °C), lowest in the larger ponds (18.5–20.4 °C) and intermediate in K and M, which partially had flowing water (22.3–24.1 °C). Temperature was inversely correlated with water volume (Spearman's rank correlation, $r_s = -0.58$, $P < 0.05$) and depth ($r_s = -0.72$, $P < 0.005$).

Water samples were preserved with a small amount of chloroform, and chemical parameters were studied with Sera[®], Aquamerck[®], and Merckoquant[®] test kits. Water in all ponds was low in ions as reflected by low conductivity values (Table 1) and was slightly acidic (pH values 5.0–7.0). Other chemical parameters were close to or below the sensitivity limit of the tests: calcium, ≤ 10 mg/l; nitrate, < 5 mg/l; chloride, < 12.5 mg/l; phosphate, < 0.25 mg/l; iron, < 0.05 mg/l;

sulphate, < 200 mg/l. Carbonate hardness and total hardness were $\leq 1^\circ$ dH in all ponds except I (2° dH), J (3° dH) and F (4° dH). Correlations of conductivity with total and carbonate hardness and with pH were significant ($r_s = 0.76–0.88$, $P < 0.005$). Such values were to be expected in the largely granite Circo de Piedrafita. The presence of high amounts of other pollutants (e.g. organic compounds) in some ponds while absent in others, is not probable in this remote and largely unaltered area. Therefore, it can be excluded with sufficient probability that the observed differences in tadpole growth rate and shape were caused by pollution (Rowe, Kinney & Congdon, 1998).

Biotic pond characteristics

Aquatic vegetation (*Sparganium angustifolium*) was present in ponds A, C, and D and was dense in pond G. Ponds F and J were vegetated at 30% and 80% of their surfaces, respectively, by *Carex fusca*. All other ponds had no aquatic or semi-aquatic vegetation.

Various methods that were dependent on the size and accessibility of the pond were used to estimate the number of tadpoles present. Direct counts were preferred where possible, but in large ponds and those with many hiding places, the number was estimated by neutral red staining (c. 5 min in 0.025–0.1% neutral red solution; see Cooke, 1976) of a large sample of tadpoles per pond and recapturing tadpoles the next day (Petersen, 1896). The dye of the tadpoles was always recognizable 1–2 days after staining and in some ponds even after 5–6 days. Tadpole mortality during the staining procedure was low and mainly caused by a lack of oxygen when too many tadpoles were stained at once. Tadpole densities/m³ were correlated with water temperature (Spearman's rank correlation, $r_s = 0.78$, $P < 0.005$) and inversely with water volume ($r_s = -0.59$, $P < 0.05$).

During nocturnal searches along the pond shores 5 different potential tadpole predators were observed: (1) newts *Euproctus asper*; (2) salamander larvae *Salamandra salamandra*; (3) dragonfly larvae; (4) *Dytiscus* beetles; (5) *Dytiscus* beetle larvae. No fishes were seen in the ponds, although 2 species occurred in the nearby dams (*Salmo trutta*, *Phoxinus phoxinus*). Aquatic shrews *Neomys fodiens* were observed sporadically in pond A but apparently did not occur in high densities. Direct observations were made of predation attempts by salamander larvae on tadpoles in pond H. The salamander larvae were not able to catch the tadpoles, which were as large or even larger than the larvae, but did succeed to bite them from behind on several occasions. The effect of these bites could not be ascertained in detail, but the larvae were probably able to remove parts of the tadpole's tails. Predator density was estimated from our nocturnal counts as specimens/m of shoreline; it ranged from 0 to 3.09/m. Numbers of salamander larvae were highest in pond H (170 specimens along the shoreline). There were no significant correlations with predator

Table 1. Main characteristics of ponds studied and selected morphological traits of the respective tadpole populations. T, water temperature; C, water conductivity; SVL, snout–vent length of tadpoles; SVL met., snout–vent length of recently metamorphosed specimens; NPAP, number of oral papillae; K1, K2, K3, number of keratodonts of first, second and third interrupted upper keratodont rows (right side of jaw sheaths only). Measurements, mean \pm standard deviation (minimum and maximum values in parentheses); developmental stages, according to Gosner (1960), mean with minimum and maximum values in parentheses; tadpole density, m³ of water; predator density, potential predator specimens/m of pond shoreline; last column, percentage of individuals with clearly damaged tails (tail tip missing or severely mutilated)

Pond	Surface (m ²)	Volume (m ³)	Tadpole density (per m ³)	Predator density (per m)	T (°C)	C (μS)	SVL met (mm)	SVL (mm)	Gosner stage	NPAP	K1	K2	K3	Mutilated tails (%)
A	9500	19000	12	0.09	19.0	40	14.54	15.4 \pm 0.14 (12.8–17.2)	37 (36–38)	85.8 \pm 10.1 (69–107)	55.0 \pm 7.8 (40–65)	29.6 \pm 11.0 (0–45)	11.3 \pm 3.5 (7–20)	0
B	1650	3150	2	0.04	18.5	12	14.13	16.35 \pm 0.98 (14.3–17.8)	37 (34–40)	101.4 \pm 12.8 (85–127)	55.4 \pm 9.72 (42–65)	30.0 \pm 9.0 (14–45)	8.9 \pm 5.2 (0–15)	27
C	1065	735	11	0.33	20.4	19	13.8	14.16 \pm 1.95 (10.3–17.2)	33 (28–36)	91.1 \pm 18.7 (69–134)	45.2 \pm 7.3 (36–60)	28.1 \pm 6.1 (16–38)	3.1 \pm 3.8 (0–10)	42
D	660	858	1	0.17	20.0	12	–	13.89 \pm 1.13 (11.9–15.6)	33 (30–35)	88.7 \pm 14.2 (65–112)	44.9 \pm 6.1 (32–54)	25.0 \pm 3.9 (19–30)	7.6 \pm 4.3) (0–15)	53
E	135	34	26	0.49	23.5	12	13.22	14.03 \pm 0.85 (12.9–15.1)	37 (35–38)	105.9 \pm 24.8 (69–160)	46.3 \pm 6.2 (36–55)	25.1 \pm 4.8 (18–35)	2.8 \pm 3.8 (0–10)	31
F	180	49	30	0.09	25.6	100	12.9	12.13 \pm 1.31 (9.6–13.5)	34 (25–41)	76.6 \pm 21.1 (26–98)	39.8 \pm 7.9 (26–50)	14.6 \pm 6.7 (5–25)	0	13
G	75	8	181	0.24	29.1	32	13.5	14.19 \pm 1.09 (12.9–16.5)	34 (31–37)	100.6 \pm 13.8 (83–126)	45.0 \pm 6.9 (35–55)	24.0 \pm 5.9 (15–30)	0.2 \pm 0.6 (0–2)	25
H	215	43	211	3.09	27.1	18	12.23	13.31 \pm 1.04 (11.2–14.6)	35 (26–41)	94.3 \pm 18.9 (63–116)	46.9 \pm 10.5 (32–65)	24.6 \pm 6.9 (14–35)	0.5 \pm 1.3 (0–4)	47
I	30	9	632	0	26.4	62	10.12	9.81 \pm 1.68 (7.5–13.0)	30 (25–41)	90.4 \pm 19.3 (65–116)	44.8 \pm 6.34 (35–50)	13.1 \pm 6.6 (3–20)	0	0
J	70	8	4	0	25.0	79	–	12.18 \pm 0.64 (11.1–13.1)	37 (36–41)	89.7 \pm 21.7 (66–130)	43.5 \pm 5.2 (40–57)	22.8 \pm 6.9 (15–35)	1.2 \pm 2.1 (0–5)	17
K	10	1	163	0	24.1	–	–	11.09 \pm 1.92 (9.9–17.5)	29 (25–32)	76.0 \pm 7.9 (65–93)	35.9 \pm 3.9 (30–43)	19.2 \pm 2.3 (16–23)	0.3 \pm 1.0) (0–3)	14
L	5	1	41	0	25.1	30	11.2	11.45 \pm 1.31 (8.4–13.2)	34 (26–36)	106.7 \pm 11.1 (76–118)	39.6 \pm 3.6 (33–45)	25.8 \pm 5.0 (16–35)	5.8 \pm 3.9 (0–10)	1
M	2	1	30	0	22.3	25	–	12.08 \pm 1.48 (10.7–14.9)	32 (29–35)	86.8 \pm 12.8 (70–111)	36.9 \pm 5.5 (31–50)	21.1 \pm 5.5 (16–30)	0	0
N	20	1	–	0.27	30.5	–	–	10.59 \pm 0.82 (9.5–12.0)	37 (37–38)	–	33.3 \pm 14.2 (15–48)	14.8 \pm 10.5 (0–24)	0	–

density of any physical or chemical variables, nor with tadpole density.

No data are available on the start of the breeding season of frogs in the different ponds in the study year. However, data from May and June 2000 showed that breeding started more or less simultaneously in different small and large ponds of the Circo de Piedrafita. Clutches were already present in some of the smaller ponds (e.g. pond I) heated by the sun, while they were laid some days later in the large pond A. Thaw most probably proceeds each year in a similar succession in the ponds of the area. It can therefore be excluded with sufficient probability that tadpoles from small ponds were consistently younger than those from the large ponds.

Test of genetic homogeneity

To verify that the morphologically different tadpoles belong to the same species (see Vences, Kupfer *et al.*, 1998), a fragment (up to 544 bp) of the mitochondrial 16S rRNA gene was sequenced in 1 specimen from ponds A–C, E–F, H–I and L–M. Primers, PCR protocols and sequencing conditions were as in Vences, Kosuch *et al.* (2000). No differences were detected among the sequences (Genbank accession numbers AF275735–AF275743) except a single substitution in the tadpole of pond C at position 208 of the alignment. Western Palearctic brown frog species differ by least 3% of the homologous gene fragment (M. Veith, J. Kosuch & M. Vences, pers. obs.). The genetic data thus confirmed that the different tadpole morphs correspond to a single species, *Rana temporaria*.

Morphological measurements

Tadpoles (10–15 specimens) were collected in each pond on 15 and 16 July 1999, anaesthetized and preserved in 4% formalin. Terminology of tadpole morphology follows Altig & McDiarmid (1999) and Viertel & Richter (1999) except for the term 'keratodont', which we use for the labial teeth of tadpoles according to Dubois (1995). The developmental stage was recorded according to Gosner (1960); snout–vent length (SVL), tail length (TL), maximum tail height (TH), maximum body width (BW), and number and arrangement of keratodont rows using the standardized formula of Dubois (1995), number of buccal papillae, and number of keratodonts in the first, second and third interrupted upper rows (K1, K2, K3) on the right side of the jaw sheaths. Morphometric measurements were taken with callipers to the nearest 0.1 mm and mouthparts were examined with a stereomicroscope. In some samples, a large percentage of tadpoles had damaged tails (compare Blair & Wassersug, 2000). This was obvious as generally the posterior part of the tail was missing to varying degrees. A specimen was scored as having a damaged tail only if the mutilation was obvious from a fresh wound or recognizable from the straight (not

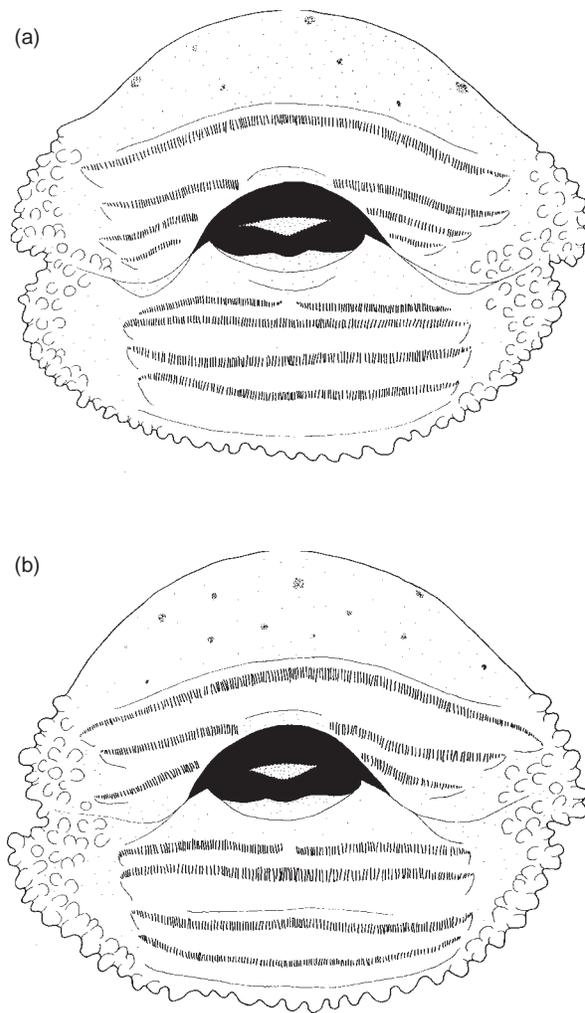


Fig. 1. Different types of oral morphology observed in *Rana temporaria* tadpoles of the Circo de Piedrafita. Note: three interrupted upper tooth rows in specimen from pond A (a), two interrupted upper tooth rows in specimen from pond I (b).

pointed) tail tip. One to 3 specimens of each sample were prepared by critical point drying and their mouthparts, especially buccal floor and buccal roof, were examined with a scanning electron microscope (SEM). All specimens (except those used for SEM analysis) are preserved in the collection of the Zoologisches Forschungsinstitut und Museum A. Koenig, Bonn, Germany (ZFMK 72115–72140). Statistical analysis was carried out with SPSS for Windows (version 9).

RESULTS

All measured morphological variables and developmental stages differed significantly among ponds (Kruskal–Wallis ANOVA, $P < 0.001$). Highly significant differences ($P < 0.001$) were found in all variables except for K3 (not significant) and K2 ($P < 0.05$) by ANCOVA with SVL as the covariate; and in all variables except K1 ($P < 0.005$) by ANCOVA with stage as the covariate.

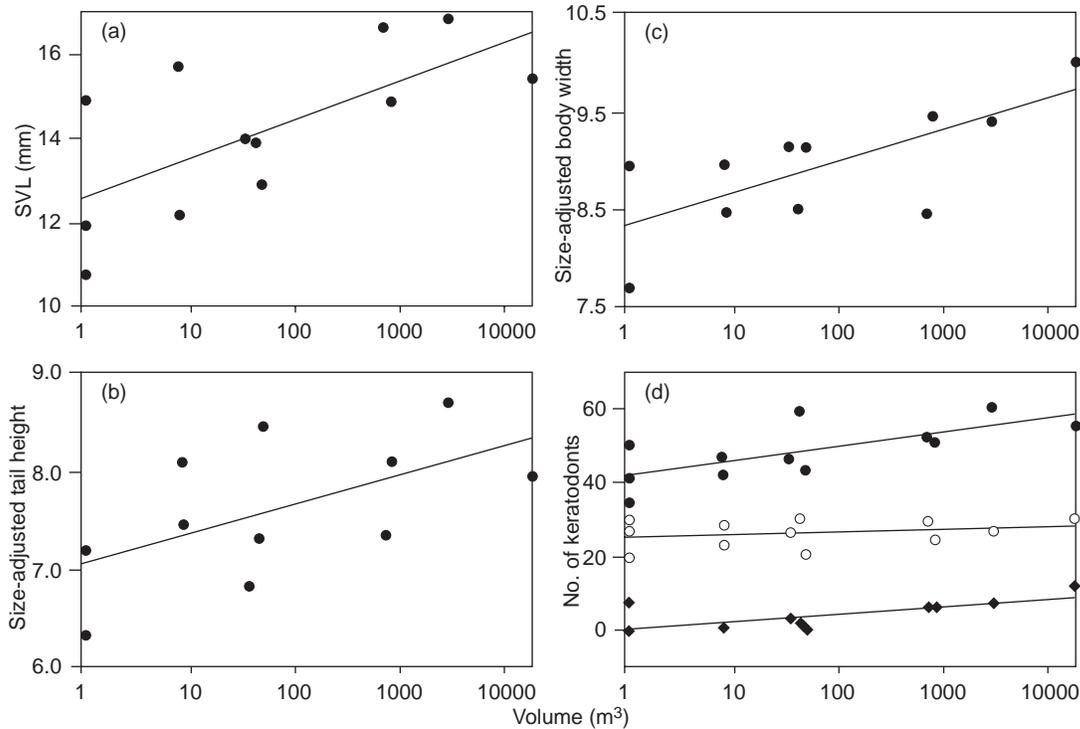


Fig. 2. Growth rate and morphological traits of *Rana temporaria* tadpoles relative to pond size. Snout-vent length (a), size-adjusted tail height (b), size-adjusted body width (c), and number of keratodonts of the upper first (●), second (○) and third (◆) interrupted tooth row (d) were plotted against water volumes (logarithmic scale). Each symbol represents mean value of one pond (only specimens in developmental stages 35–38 considered). Tail height and body width were adjusted to the mean overall SVL of 12.99 mm. Correlations were significant (Spearman's rank correlation, $P < 0.05$) in A, C, and D, as well as for total number of keratodonts and keratodonts on first interrupted upper row in B.

Tadpole size differences between ponds were very conspicuous; mean SVL in pond B was almost twice the value in pond I. Tadpole keratodont formulas differed in the number of interrupted keratodont rows on the upper lip. These were three (resulting in a formula of 1:3 + 3/1 + 1:3) in almost all specimens of the three largest ponds (A, B, D), in about half of the specimens in the fourth largest pond (C), and in most specimens of pond L. Tadpoles in the remaining ponds had exclusively or mostly only two interrupted upper rows (formula 1:2 + 2/1 + 1:3) (Fig. 1). SEM pictures did not reveal consistent differences in size and arrangement of the papillae of the buccal floor and buccal roof, which corresponded well to the drawings of Viertel (1982). All specimens had four lingual papillae; in several individuals, one to three of these were damaged (only their bases visible) and difficult to recognize.

Mean Gosner stage was not significantly correlated with water volume, temperature, nor tadpole density. Metamorphosis occurred in nine ponds during the study period. The date of first metamorphosis observation (developmental stages 41–46) was 14 July in pond F, 21 July in ponds E and I, 22 July in pond B, 25 July in pond A, and 28 July in ponds C, G, H, and L and was not correlated with water volume, temperature, or tadpole density. Mean SVL of the metamorphosing specimens (up to five individuals measured) was positively correlated with water volume ($r_s = 0.75$, $P < 0.05$),

and negatively correlated with tadpole density ($r_s = -0.90$, $P < 0.005$) but not with temperature.

To exclude the effects of stage differences on the morphometric variables, mean values per pond were calculated based on specimens in stages 35–38 only. No such specimens were present in the samples of ponds I and K; the analyses therefore were carried out with a reduced data set of 11–12 ponds. Numbers of upper keratodont rows were similar to the overall sample. In the four large ponds the percentage of specimens with three upper keratodont rows was 66–80% (ponds A, B and D) and 50% (pond C). In pond L, 92% of the tadpoles had three rows while the percentages were 0–35% in the remaining small ponds. SVL (as most other morphological measurements) was correlated with water volume ($r_s = 0.60$, $P < 0.05$). Mean number of keratodonts of all three rows studied increased with water volume, but the respective correlation was only significant for K1 ($r_s = 0.74$, $P < 0.01$). A significant correlation was also found for the total number of keratodonts, when all three rows were added together ($r_s = 0.65$, $P < 0.05$; Fig. 2). No obvious differences in keratodont shape or size were noted in the SEM analyses. Similar (but inverse) correlations and significance levels were found between morphology and water temperature but not with tadpole density. No significant correlation of any environmental variable was noted with number of papillae.

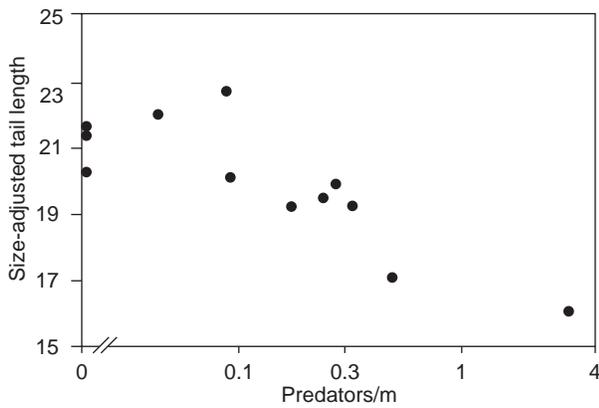


Fig. 3. Size-adjusted tail length of specimens with apparently intact tails plotted against predator density (individuals counted per m of pond shore). Each symbol represents mean value of one pond. Values are based on specimens in stages 35–38 only. Predator density, semi-logarithmic scale to account for the high values in pond H. The correlation was significant (Spearman's rank correlation, $P < 0.005$).

To exclude the size factor from the morphometric measurements TL, TH and BW, we log-transformed these variables, and subsequently removed $\log(\text{SVL})$ as a covariate through regression analysis. Values were adjusted to the overall mean SVL (12.99 mm); thus, all specimens were compared as if they had this average SVL (Blouin, 1992). Size-adjusted tail height was correlated with water volume ($r_s = 0.648$, $P < 0.05$) and inversely with tadpole density ($r_s = -0.651$, $P < 0.05$). Size-adjusted body width was positively correlated with volume ($r_s = 0.680$, $P < 0.05$) and negatively with tadpole density ($r_s = -0.647$, $P < 0.05$) and temperature ($r_s = -0.618$, $P < 0.05$). Size-adjusted tail length of specimens with apparently non-damaged tails was not correlated with volume, temperature and density, but was negatively correlated with predator density ($r_s = -0.831$, $P < 0.005$; Fig. 3). The percentage of damaged tails among complete samples of all ponds was positively correlated with predator density ($r_s = 0.727$; $P < 0.01$) but not with any other environmental factor.

DISCUSSION

Modifications of tadpole growth rate and size at metamorphosis by environmental factors are of two sorts. With increasing temperature (Harkey & Semlitsch, 1988) or pond desiccation rate (e.g. Newman, 1989; Denver, Mirhadi & Phillips, 1998; Loman, 1999) time to metamorphosis may be reduced and metamorphosis occurs at smaller or similar body sizes. Alternatively, time to metamorphosis is prolonged, and metamorphosis nevertheless occurs at smaller sizes; this response has been observed at lower food resources or higher density (e.g. Gromko *et al.*, 1973; Miranda & Pisanó,

1993; Reques & Tejedo, 1995). According to Denver (1997), the same effects may be inhibitory to growth if present early in the larval phase, but stimulatory to development if present during prometamorphosis. *Rana temporaria* tadpoles at the Circo de Piedrafita probably experienced the relevant environmental influences (differences in pond size, density and temperature) upon hatching. They grew slower and metamorphosed at a smaller size with decreasing pond volume (and increasing temperature and density), although no significant modification of time to metamorphosis was noted. The observed pattern seems to match better the second category outlined above. Which factor or combination of factors is most responsible for the growth differences is difficult to assess without controlled experiments, considering their strong inter-correlations. As the ponds studied were poor in minerals and algae it could be assumed that food was a limiting resource in ponds with high density, accounting, partially at least, for the reduced growth.

The factors that account for differences in growth rate and size at metamorphosis among tadpoles in the Circo de Piedrafita ponds are also likely to have effects on oral structures. The upper keratodont rows in *Rana* appear centripetally (Grillitsch & Grillitsch, 1989), as in other anurans (e.g. *Hoplobatrachus tigerinus*; Dutta & Mohanty-Hejmadi, 1984). This means that the outer rows appear first, while the rows closest to the jaw sheaths sequentially form at later stages. Length of rows and keratodont number increase during anuran development (Tubbs *et al.*, 1993). Specimens in earlier developmental stages thus have a lower number of keratodonts and keratodont rows. Among the surveyed ponds, however, such differences were also obvious in specimens of similar stages. It is assumed that heterochronic developmental shifts were responsible for these observations; progress of development, and eventually metamorphosis, being induced before the oral structures are fully developed. This hypothesis most probably explains why growth-inhibited tadpoles in the small ponds had few keratodonts in late stages.

Tadpoles in the small ponds also had relatively lower tail fins and their bodies were narrower. Almost 50% of the dry weight of a *Rana* tadpole is stomach content (Calef, 1973). Our field impressions were a general lower body volume in specimens from small ponds as compared to the almost spherical bodies of tadpoles found in large ponds. A lower food intake or weight loss (either due to lower food availability or related to crowding stress or pathogen factors; Gromko *et al.*, 1973; Beebee, 1995; Petranka, 1995) in the smaller ponds may therefore account for the variation in body width. The differences in tail height may be related to the long swimming distances covered each day by tadpoles in the large ponds, which migrated each day from the deep part of the ponds to the shallow edges, probably due to thermoregulatory behaviour patterns (Piqué *et al.*, 2000).

A further, independent modification of tadpole shape was caused by predation. Predators caused a higher

proportion of damaged tails, and a lower relative tail length in specimens with apparently intact tails. Most probably, these specimens were attacked and damaged by predators early in development and their tails grew back completely, albeit shorter. This observation would be in accordance with the hypothesis that tail loss in anuran larvae incurs little cost and therefore may be an important mechanism to reduce the effect of predation (Wilbur & Semlitsch, 1990).

Altogether, the morphological differences observed between ponds approach a magnitude generally considered as diagnostic between brown frog species (e.g. Barbadillo *et al.*, 1999). Together with the high degree of damage of lingual papillae detected by the SEM studies, they are in the range of the differences found by Vences, Kupfer *et al.* (1998) among Pyrenean *R. temporaria* populations. Thereby, our results do not support the hypothesis that more than one *temporaria*-like *Rana* species occurs in this mountain chain.

A keratodont formula with only two interrupted upper rows seems to be typical for montane *R. temporaria* populations (Sperling, Vences & Böhme, 1996; this study). These populations furthermore differ by accelerated growth rates from lowland populations (Brand & Grossenbacher, 1979) as is also found in other anurans (Gollmann & Gollmann, 1996). Growth rate is also higher in *R. temporaria* populations from northern latitudes as compared to those from more southern sites (Merilä *et al.*, 2000). Relative hindlimb length of metamorphs may differ relative to size at metamorphosis in frogs, caused by heterochronic effects (Emerson, Travis & Blouin, 1988). The variation in larval growth history detected in the present study certainly influences life history and survival of metamorphs. Additionally, its magnitude also makes an influence on adult morphology conceivable. If such influences were demonstrated by further studies, they may partly explain the extraordinary variability of external morphology, both within populations and between high- and low-altitude populations of *R. temporaria*.

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