CANALIZATION OF SIZE AT METAMORPHOSIS DESPITE TEMPERATURE AND DENSITY VARIATIONS IN *PELODYTES PUNCTATUS*

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We investigated variation in length of larval period and size at metamorphosis in *Pelodytes punctatus* in response to temperature and density. Experiments followed a 3 × 2 factorial design, with density and temperature as fixed factors. Length of the larval period was very sensitive to temperature. Although significant, size at metamorphosis showed less variation. Density did not strongly affect the studied variables. No trade-off between length of larval period and size at metamorphosis was detected, even when a trend was perceptible at low density. *Pelodytes punctatus* differs from most other European species of tailless amphibian with regard to these traits, but is similar to *Bombina variegata*. These two species both belong to taxa of ancient origin, but also use habitats in the early stages of ecological succession (e.g. those following physical disturbance). Further studies are required to distinguish the influence of phylogenetic factors from those ecological factors that drive the evolution of such biological traits.

Key words: larval period, plasticity, size at metamorphosis, tadpole development

INTRODUCTION

Phenotypic plasticity is a response to environmental variability. Plasticity may be adaptive when an animal is faced with immediate constraints, but it can also respond to habitat unpredictability by producing phenotypic variation within the progeny (Kaplan, 1987; Scheiner, 1993; Schlichting & Pigliucci, 1995). In animals with complex life cycles, the timing of the transition from one biological stage to another through metamorphosis would ideally be optimized by an accurate assessment of the conditions in the premetamorphic and postmetamorphic habitats respectively (Wilbur & Collins, 1973; Wilbur, 1980; Werner, 1986; Alford & Harris, 1988). However, only information on the immediate habitat is available to the individual. As a consequence, the timing of metamorphosis depends on information gathered in the premetamorphic habitat and putative performance objectives in the postmetamorphic habitat that have been forged by natural selection. Such performances are mainly influenced by body size, and consequently metamorphosis is expected to be delayed until body size reaches a specific threshold that optimizes the trade-off between growth in the premetamorphic and postmetamorphic habitats (Wilbur & Collins, 1973; Werner, 1986). As a consequence, body size at metamorphosis, length of the larval period, and survival may vary according to premetamorphic ecological conditions. However, such a trade-off (negative correlation) between the shortness of the larval period and the size at metamorphosis is expected to follow species-specific reaction norms, as it results from specific interactions between habitat and genotype.

In tailless amphibians, variation in the timing of metamorphosis has been well documented. Most studies have shown that growth rate and body size determine the timing of metamorphosis according to threshold effects (Wilbur & Collins, 1973; Smith-Gill & Berven, 1979; Alford & Harris, 1988; Hensley, 1993). The factors that most strongly influence size at metamorphosis are density, food availability, temperature and stress (Travis, 1984; Berven, 1987; Tejedo & Reques, 1994; Newman, 1994; Denver, 1997; Denver *et al.,* 1998). Moreover, comparisons of tadpole development under standardized rearing conditions among species have revealed variation in the reaction norm of the trade-off between size at metamorphosis and shortness of larval period in response to temperature treatments (Blouin, 1992 ; Morand *et al.*, 1997). In the study of Morand *et al.* (1997) that compared development of species belonging to the anuran assemblage of the Rhone floodplain, most species exhibited such a trade-off. *Bombina variegata* was an exception, and showed a canalization of size at metamorphosis (absence of trade-off as the slope of the bivariate reaction norm did not differ from zero). This result was surprising because this species mainly occupies unstable habitats within floodplains (Morand, 1996). Such habitat use would be expected to promote plasticity.

The aim of the present study was to firstly investigate reaction norms to temperature and density in *Pelodytes punctatus*, a species that also inhabits unstable habitats in the early stages of ecological succession (i.e. 'young' habitats that appeared recently or that experience frequent physical disturbances that maintain them at early successional stages). These habitats are often located within floodplains (Diaz Paniagua, 1988; Toxopeus *et al.*, 1993; Morand, 1996). Secondly, the life history of this species is poorly documented and the present study also aimed to provide precise estimations of length of larval period and size at metamorphosis under controlled conditions.

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TABLE 1. Mean survival, length of larval period (±SE), body length (±SE), and dry mass (±SE) of *P. punctatus* for each replicate according to temperature and density.

Density	Replicate	Survival (% metamorphs)		Length of larval period (days)			Dry mass (mg)		
		15°C	25°C	15°C	25°C	15°C	25°C	15°C	25°C
5	1	100	80	100±15	31±3	18.8±0.8	16.5±1.2	98.1±22.4	55.1±14.4
5	2	100	60	89±10	46±6	18.5±1.0	16.3±0.6	107.6±17.0	55.4±6.1
5	3	80	80	103±19	41±7	17.3±0.9	16.5±1.1	70.1±16.5	64.3±8.8
	Mean	93	73	97.3	39.3	18.2	16.4	91.9	58.3
15	1	73	60	76±5	54±9	17.3±1.2	16.7±1.3	72.2±9.6	69.6±14.1
15	2	67	80	89±18	58±12	15.9±1.4	15.5±1.1	54.7±19.1	47.8±11.2
15	3	60	100	104±22	65±11	15.8±0.9	15.3±0.9	48.8±7.5	45.9±12.9
	Mean	67	80	89.7	59.0	16.3	15.8	58.6	54.4
30	1	50	33	106±13	61±16	15.8±1.3	16.1±0.6	50.4±14.9	54.3±5.0
30	2	53	33	93±16	72±24	15.6±1.2	15.7±0.8	47.3±13.5	50.0±7.5
30	3	77	60	115±10	58±19	16.5±0.8	14.5±0.8	56.5±9.5	37.6±6.4
	Mean	60	42	104.7	63.7	16.0	15.4	51.4	47.3

MATERIAL AND METHODS

Three clutches of eggs were collected in April 1996 in the Jons sector of the Rhone floodplain (see Joly, 1992). After hatching, tadpoles were distributed randomly among 18 tanks according to a 3 × 2 factorial design. Density and temperature were fixed factors with three levels of density (5, 15 and 30 tadpoles per tank) and two levels of temperature (15 and 25 °C). Each treatment (density × temperature) was replicated three times. Replicates were distributed randomly in the experimental room. Each tank was 40 × 40 cm long and 10 cm deep, and contained 4 l of continuously aerated water. The lower temperature (15°C) was the ambient temperature in the cooled room, whereas the higher temperature (25°C) was maintained by using water heaters in the aquaria. Temperature was checked in each tank three times a week, and tadpoles were counted and the tanks were cleaned. Boiled lettuce was provided *ad libitum* as food.

When the froglets reached metamorphosis, they were humanely euthinased, then dried on filter paper, measured to the nearest 0.1 mm (snout-urostyle length) under a stereoscopic microscope and weighed to the nearest 1 mg. Dry mass was measured after heating for 48 hr at 80°C. Survivorship was estimated by the number of tadpoles that successfully reached metamorphosis. After experimental groups were selected, surplus tadpoles were carefully reared in large tanks and released at the sampling site when they reached stages 35-40 (Gosner 1960) to compensate for sampling impact on the source population.

Survivorship, size and mass at metamorphosis, and length of the larval period were compared by a two-way ANOVA. Before performing ANOVAs, data were checked for homogeneity of variance and normality. Survival data were transformed from simple proportions (p) to arcsin ($\sqrt{p}$) before analysis. Pairwise comparisons of treatment means were performed using Scheffé's tests.

RESULTS

Survival to metamorphosis, mean length of larval period, mean body length and mean dry mass in each replicate are listed in Table 1. Mean survival to metamorphosis varied with density ($F_{2,12}=6.40$, $P=0.013$), declining as density increased, but it did not differ significantly between temperature treatments (Fig. 1a, Table 2).

The mean length of larval period was significantly greater at 15°C than at 25°C at all densities (Fig. 1b). Length of larval period also increased with increasing density; this trend was more apparent at 25°C than at 15°C ($F_{2,12}=3.43$, $P=0.066$, Table 2).

Both body length and dry mass at metamorphosis were affected by the temperature and density treatments (Figs 1c and 1d ; Table 2). Body length and dry mass differed significantly between temperature treatments, but only at low density. On day 5, mean body length and mean dry mass were higher at 15°C than at 25°C (Table 1; Fig. 1) (Scheffé tests; $P=0.032$ and $P=0.014$, respectively; for the other densities: $P>0.05$). Density affected body length and dry mass only at low temperature, with tadpoles larger at lower density (Scheffé tests on 15°C data; for body length: days 5-15, $P=0.023$; days 5-30, $P=0.008$; for dry mass: days 5-15, $P=0.015$; days 5-30, $P=0.004$). No difference was detected between day 15 and day 30 (Scheffé test; $P>0.05$). At 25°C, size at metamorphosis was unaffected by density (Scheffé tests; $P>0.05$).
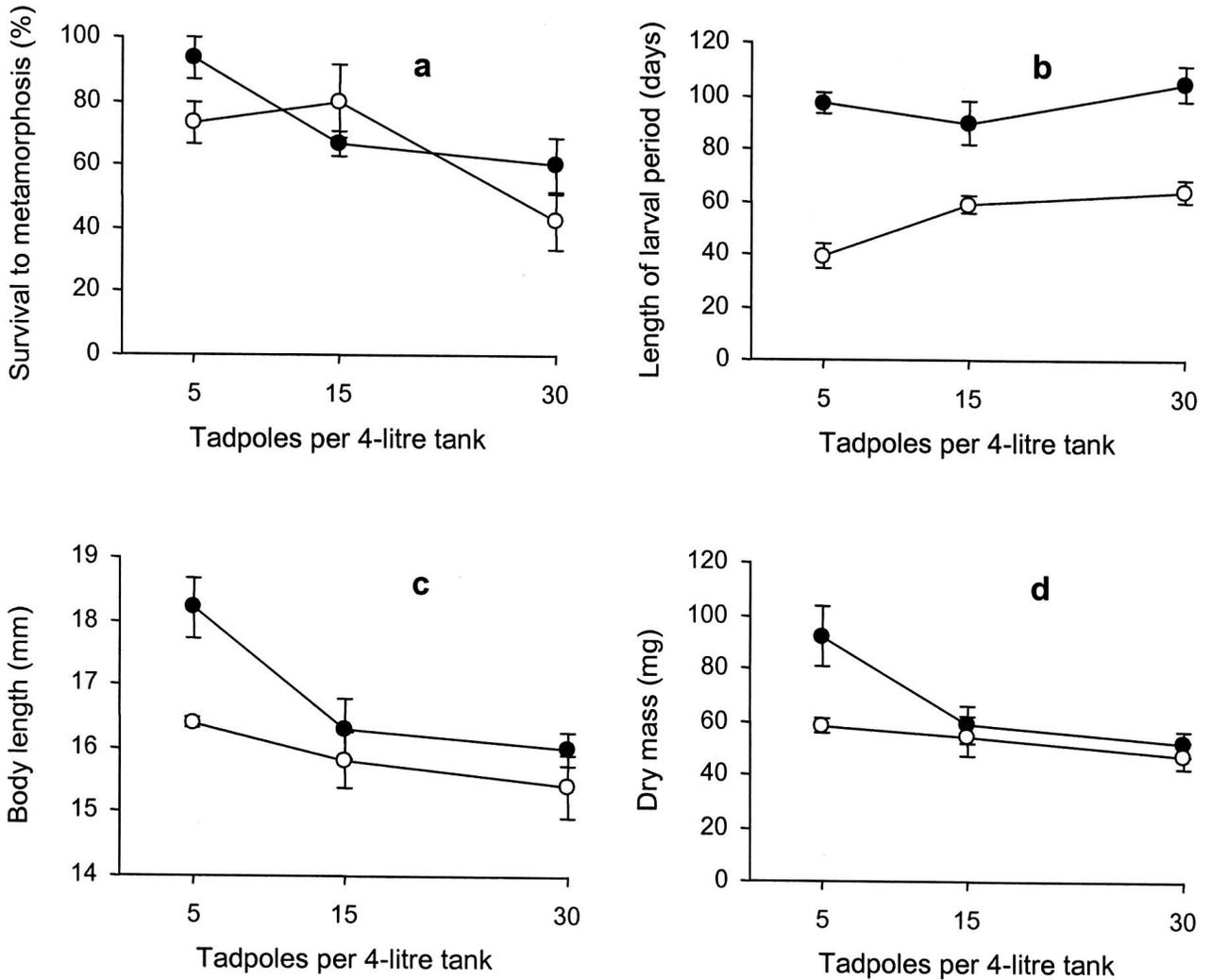
FIG. 1. Influence of temperature and density on (a) survival; (b) length of larval period; (c) body length and (d) dry mass. Solid squares: low temperature (15°C); open squares: high temperature (25°C).

TABLE 2. ANOVAs for length of larval period, body length and dry mass of *P. punctatus* tadpoles at metamorphosis. ***$P<0.0001$, **$P<0.001$, *$P<0.05$.

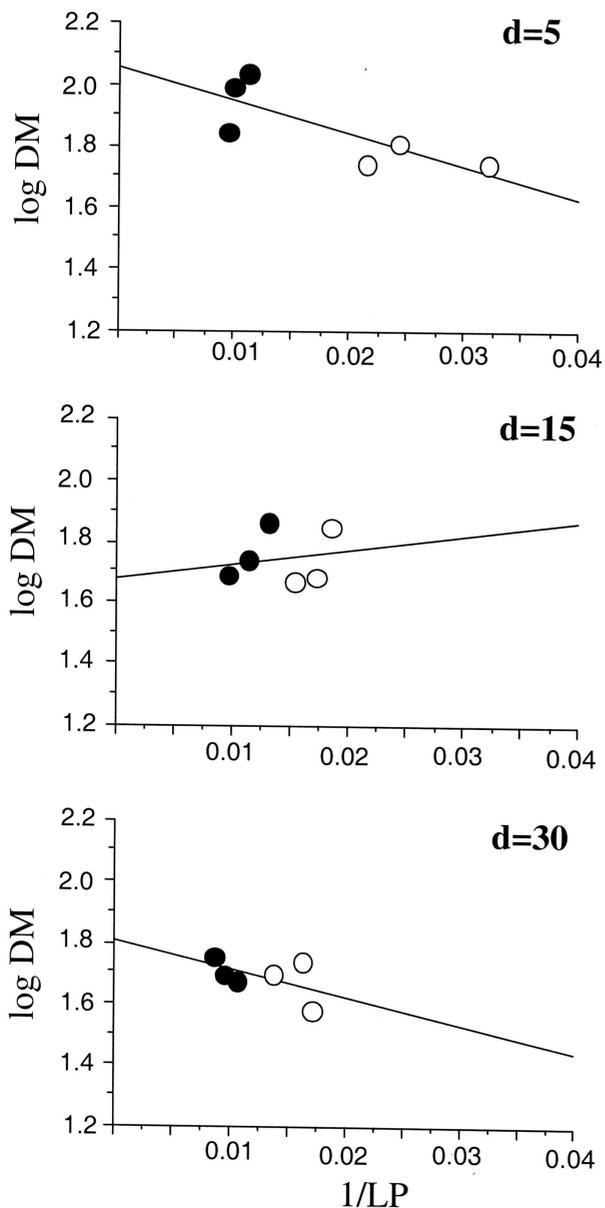|	Source of variation	df	MS	P
Length of larval period	Temperature	1	8390.76	98.59***
	Density	2	380.28	4.47*
	Temp. × Density	2	292.15	3.43
	Error	12	85.11	1.85
Body length	Temperature	1	3.80	7.82*
	Density	2	4.23	8.68*
	Temp. × Density	2	0.76	1.55
	Error	12	0.49	
Dry mass	Temperature	1	877.90	6.38*
	Density	2	1057.89	7.69*
	Temp. × Density	2	435.49	3.16
	Error	12	137.62	

FIG. 2. Bivariate reaction norms of size at metamorphosis (log (dry mass)) and length of larval period (1/LP) to temperature for each density. Full dots: individual data at 15°C; open dots: individual data at 25°C. DM = dry mass; LP, larval period; d, density. Dry mass appeared to be influenced by temperature only at low density.

The bivariate reaction norms of dry mass in relation to length of larval period (1/length of larval period) highlighted the absence of trade-offs (negative correlations) at each density (P=0.06 for day 5; P=0.71 for day 15; P=0.27 for day 30; Fig. 2). However, the P-value for day 5 was close to significance, suggesting a trend toward a trade-off at this density level.

DISCUSSION

Our data can be compared with those from other species belonging to the anuran assemblage of the Rhone floodplain obtained by Morand *et al.* (1997) with the same rearing equipment (Table 3). As density was 30 tadpoles per 4 l in these previous experiments, comparisons are possible at this density. Length of larval period of *Pelodytes punctatus* was one of the longest of the species studied, and was very close to that of *Bombina variegata* at both low and high temperatures. This result differs from that of Diaz Paniagua (1988) who considered that development of *P. punctatus* (approximately 80 days in the field) was as short as that of other species. However, the latter study concerned length of larval period under field conditions where *P. punctatus* did not spawn at the same time of the year as most other species, making comparisons among species less straightforward because of seasonal variation in temperature, food availability and competition. In the north of France, Toxopeus *et al.* (1993) also found the duration of the larval period varied from 67 to 102 days in the field, depending on the year. From other studies, the length of the larval period in the field ranges from 73 to 97 days (Balcells, 1955; Girard, 1989). All these data are within the range we observed (from 31 to 115 day s depending on both temperature and density). We are confident in our comparisons with other species studied by Morand *et al.* (1997) because of similar controlled conditions. When compared to the data of Morand *et al.* (1997), size at metamorphosis of *P. punctatus* reached high values (Table 3). This result is consistent with that of Diaz Paniagua (1988), who also showed that premetamorphic tadpoles of *P. punctatus* reached a larger size than in most other species, except for *Pelobates cultripes*, the tadpoles of which are exceptionally large.

TABLE 3. Comparison of larval traits of *Pelodytes punctatus* with those of other European species reared at two temperature levels and at a density of 30 tadpoles per 4 litres. Data from Morand *et al.*, 1997.

Temperature	Dry mass (mg)		Length larval period (days)	
	15	25	15	25
Pelodytes punctatus	51±4	47±8.7	104±11	63±7
Bombina variegata	25±2	25±1	106±4	65±4
Bufo calamita	14±1	7±0.4	73±1	44±2
Bufo bufo	30±1	9±0.3	73±1	29±0.3
Rana temporaria	41±2	24±3	64±1	48±3
Rana dalmatina	60±3	25±2	106±2	49±7

Long larval period and large size at metamorphosis often correspond to the use of permanent water habitats (Pough & Kamel, 1984). However, *Pelodytes punctatus* is not a typical inhabitant of permanent waters, as most studies state that this species avoids permanent waters where fish are present. However, it can be abundant in permanent waters where fish are absent, such as gravel pits (Morand, 1996) or dune ponds (Toxopeus, *et al.* 1993). It also seems to avoid competition with other species by occurring in mesotrophic and early succession habitats that can be temporary (Diaz Paniagua, 1988; Morand, 1996; Piégay *et al.*, 1997). Morand *et al.* (1997) also noted a long larval period in *Bombina variegata* which occupies early succession habitats that can be temporary (e.g. water-filled ruts and small ponds that are frequently flooded).

In comparing different temperature conditions, a slight trade-off between shortness of larval period and size at metamorphosis was only detected at low density. High temperatures are usually expected to induce a shortening of larval period and hence a lower size at metamorphosis (Blouin, 1992 ; Morand *et al.,* 1997). However, canalization of size at metamorphosis with respect to temperature variation has already been shown in a few species such as *Hyla squirella* (Blouin, 1992) and *Bombina variegata* (Morand *et al.* 1997). As for long larval periods and large size at metamorphosis, low plasticity of size in response to temperature variation is a biological trait shared by both *P. punctatus* and *B. variegata*, but not by other European anurans. Demonstrating the adaptive value of this trait is not an easy task because confounding factors such as phylogeny can influence the expression of biological traits (Harvey & Pagel, 1991). In this context, both species belong to ancient groups of tailless amphibians (i.e. Archeobatrachia for *B. variegata* and Mesobatrachia for *P. punctatus*). Other species belonging to these old groups, such as *Alytes obstetricans* (Guyétant, 1975) and *Pelobates cultripes* (Knoepffler, 1961; Diaz Paniagua, 1988), also show large size at metamorphosis and long larval periods.

According to Werner (1986), the evolution of size at metamorphosis results from a trade-off between performance in aquatic habitats and performance in terrestrial habitats, before and after metamorphosis, respectively. Canalization of size at metamorphosis suggests that success in the terrestrial habitat is constrained by selective pressures for a given minimum size. Such pressures remain to be identified since our knowledge of the ecology of juvenile anurans is still scanty. However, it is surprising that the species that present the highest canalization of size at metamorphosis are also those that inhabit unstable (i.e. temporary, frequently flooded) habitats where they appear as pioneer species.

Our work investigated plasticity by varying temperature, which is an important ecological factor because of its influence on development rate and on species distribution. Other factors have also to be investigated, such as water duration or presence of predators that can appear more relevant with respect to performance in the field. Other experiments in more complex environments (mesocosms) are needed to confirm our conclusions as variation in tadpole growth may be an artefact of the rearing conditions in small tanks (Richards, 1958; Biesterfeldt *et al.*, 1993). Moreover, we also need field studies that can quantitatively estimate the respective impacts of different factors on tadpole growth and survival in various habitats. Understanding the determinants of the trade-off between length of larval period and size at metamorphosis needs more observations on other species to estimate the respective influences of phylogeny (improving our knowledge of larval traits of both Archeobatrachia and Mesobatrachia) and ecology (species inhabiting unstable *vs* stable habitats).

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