Biological factors controlling developmental duration, growth and metamorphosis of the larval green toad, *Bufo viridis viridis*

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Abstract The present study in a controlled laboratory setting provided important insights into both the degree of plasticity and the proximal environmental cues operating in the response of green toad tadpoles to pond drying, food level. It was concluded that timing of metamorphosis and size at metamorphosis were highly affected by pond duration. The effects of pond desiccation are reflected by shorter developmental duration and smaller size at metamorphosis as a result of increased crowding in the shallow tanks than tadpoles in the deep tanks. *Bufo viridis* raised on high food supplements grew faster than those raised on low food in low or high population density. In the tanks with decreased water and food levels, the tadpoles accelerate development and metamorphose earlier than tadpoles in higher food and water levels. The obtained data revealed that tadpoles grew faster under conditions of high population density than low one in either high or low food levels. Actual density had limited but significant effects on tadpole size and development. It also suggested that density regulation, acting on the tadpole stage, may be present in the population but was of less short-term importance than abiotic factors. Environmentally induced variation in developmental rates translated to changes in relative hind leg length. Hind leg length plasticity was positively correlated with growth rate plasticity. Finally, documenting the recent results of this study, *B. viridis* breed in temporary ponds and exhibited plasticity in developmental duration and growth rate in response to a change in water level.

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Introduction

Growth rate and metamorphosis of many aquatic organisms may vary with changing physical and chemical conditions of the surrounding medium. Anuran species with aquatic herbivorous larvae that metamorphose into terrestrial carnivorous juveniles are classic examples of animal species with complex life cycles. The ecological cues associated with the initiation of a change in stage remain unclear (Werner,

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1986). Wilbur and Fauth (1990) proposed a model that predicts the timing of amphibian metamorphosis; Travis (1984) subsequently proposed a simpler alternative model for anuran metamorphosis. The two models produce similar correlations of body size at metamorphosis with length of larval period. The Wilbur–Collins and the Travis hypotheses differ fundamentally with respect to the plasticity of larval developmental and growth rates. The Wilbur–Collins model predicts that developmental rate responds to changes in growth rate throughout the larval period after a minimum size has been attained. They also suggest that rapidly growing amphibian larvae above a minimum size might metamorphose when growth rate dropped below a threshold value, in an adaptive response to decreased food availability. Their model also predicts that metamorphosis should be delayed if growth is consistently slow. The Travis model predicts that developmental rate is set early in the larval period. Subsequent changes in growth rate would affect the size of the body at metamorphosis but not the length of the larval period. Thus, the proportion of the larval period during which individuals can respond to changes in growth rate by altering developmental rate is the fundamental difference between the two models. Because of the high morphological variability of the green toad, several forms, as species and subspecies, have been described within its extensive range (Stock et al., 2001). In Italy seems to be present the nominate subspecies (Bologna and Giacoma, 2006); however, the taxonomical status of the green toad has not so far been clearly assessed, and further investigations are needed (Roth, 1986; Werner, 1994; Balletto et al., 2000; Stock et al., 2001; Stok et al., 2006; Bologna and Giacoma, 2006; Sicilia, 2006). Werner (1986) developed a model for amphibians predicting optimal size at metamorphosis for habitats characterized by growth opportunity and risk of mortality. However, many habitats vary in quality from pond to pond or during the larval period (Semlitsch and Caldwell, 1982; Travis, 1984), and a larva that responds appropriately to different conditions may have higher fitness than one with fixed size at metamorphosis or fixed length of larval period. Plasticity in development may be adaptive in a variable environment (Casewell, 1983; Lively, 1986). For example, Alford and Harris (1988) demonstrated that growth history in *Bufo woodhousei fowleri* does affect timing of metamorphosis. Denver et al. (1998) demonstrated the adaptive plasticity in amphibian metamorphosis and reported that the lower and upper limits to the length of the larval period and body size at metamorphosis are central amphibian life history traits (Alford, 1999; Smith, 1987). Phenotypic plasticity is the ability of a single genotype to produce alternative morphologies, physiological states or behaviours in response to different environmental regimes (Gilbert, 2003; West-Eberhard, 2003). Phenotypic plasticity thus refers to the flexible response of a genotype to variety in the environment (Schlichting and Pigliucci, 1998). Inducible plasticity is defined as phenotypic changes in response to an external environmental change (Pfennig, 1992; Gilbert and Schreiber, 1995, 1998; Tollrian, 1995; McCollum and Leimberger, 1997; Slusarczyk, 1999; Michimae and Wakahara, 2002; Kishida and Nishimura, 2005). Body size is positively correlated with survival and fecundity and associated with many of the most fundamental processes of biology: metabolism and movement (Schmidt-Nielsen, 1984; Brown et al., 2004), rates of reproduction (Peters, 1983) evolution (Allen et al., 2006) and the likelihood

of extinction (Gaston and Blackburn, 1995). A larger size often requires additional time for growth, and results in an older age at metamorphosis (Merilä et al., 2004; Lardner, 2000; Loman and Lardner, 2009). Size-related properties can also affect responses to climate change (Gardner et al., 2011) as well as alter food web structure and dynamics (Brose, 2010; Thierry et al., 2011). The role that plasticity, induced during the larval phase, may play in driving adaptive divergence (e.g. through genetic assimilation) deserves more research (Gomez-Mestre and Buchholz, 2006; Wund et al., 2008). Plasticity of shape in juvenile frogs across environments is dependent on variation in either developmental and/or growth rate plasticities. Theoretical studies suggest that the optimal timing of metamorphosis is based on maximizing growth and minimizing mortality in both the pre-metamorphic and post-metamorphic stages, or balancing the costs of a smaller body size against the risks of an older age at metamorphosis. In anurans, size at metamorphosis may affect juvenile physiology or performance (Pough and Kamel, 1984; Taigen and Pough, 1985; John-Alder and Morin, 1990; Newman and Dunham, 1994; Gotthard and Nylin, 1995; Abrams et al., 1996; Rudolf and Rödel, 2007), survivorship (Pfennig et al., 1991), and size, age, and reproductive success at maturity (Berven, 1982, 1990; Smith, 1987, 2005; Semlitsch, 2002; Semlitsch and Wilbur, 1988). According to Degani et al. (2012) Plasticity in amphibian species, which breed in extreme conditions at the southern frontier of their distributions, allows an individual to prolong the larval period and maximize its size at metamorphosis when conditions are favourable. Plasticity may allow tadpoles to avoid mortality in a desiccating habitat by accelerating metamorphosis and reducing their size at metamorphosis.

Aim of the work

Little is known about the ecological interactions of *Bufo viridis*, so the aim of the present study is to investigate the effects of pond duration or water volume, food levels, crowding, and interspecific competition on *B. viridis* tadpoles and to test their ability to undergo phenotypic changes in larval size and the course of metamorphosis relative to time by manipulating these aspects of the environment that accompany habitat resources and monitoring fitness measures (survival, growth rate, and development) of larval anurans. In addition, it is an attempt to understand the mechanistic bases of developmental processes in an ecologically relevant context which is valuable to the elucidation of constraints on the coevolution of mechanisms controlling growth and differentiation.

Material and methods

The study site (Fig. 1) is Burg El-Arab region, 50 km west of Alexandria on the northwestern coastal region of Egypt at 31.3° latitude and 30.1° longitude (UNESCO, 1977). The anuran species used in this study was the green toad, *B. viridis* (Laurenti, 1768) and the Egyptian *Bufo regularis* (Reuss, 1834). *B. viridis* (Laurenti, 1768) is a widespread species with a range which extends from eastern France and Italy to central Asia, including northern Africa and numerous Mediterranean islands (Bologna and Giacoma, 2006) (Fig. 2, left). The Egyptian toad, *B. regularis* is a

Fig. 1 A location map of the study site. The study site is Burg El-Arab region, 50 km west of Alexandria on the northwestern coastal region of Egypt at 31.3° latitude and 30.1° longitude.

semi-aquatic common water toad found in farmed areas. (Fig. 2, right) (Hussein and Darwish, 2000). It is easy to differentiate between the tadpoles of the two species where the tadpoles of the green toad *B. viridis* are light grayish or green while tadpoles of the Egyptian toad, *B. regularis* are dark brown to black with very obvious nostril openings. Also the tadpoles of *B. viridis* are obviously taller (about 1.5 times taller) than tadpoles of *B. regularis* at the same stage of development (Fig. 3) but with more abundance of *B. viridis* tadpoles which are used as the primary studied animals in this study while *B. regularis* tadpoles are used only as competitors (Hussein and Darwish, 2000).The spawning occurs in March in a diverse range of water bodies including temporary ponds that are sporadically filled by rain, swamps, stream- and river pools, reservoirs, ditches

and puddles, as rule not deeper than 50 cm. The spawn is deposited in two strings of 2–7 m length. Larval development and Metamorphosis occur from spring through the summer (Degani et al., 2007; Hussein, 1995). The tadpoles have Labial teeth row formula (LTRF) as 2/3 with gab in A-2 that is typical for Bufonidae in general (Bekhet, 2012) (Figs. 4a and 4b). Young tadpoles from the two species in approximately the same stage of development, larvae ≈30 h old, just prior to breaking free from jelly, stage 20, after Sedra and Michael, 1961, were collected from the same locality from four egg clusters from a single natural pond at the study area in Burg El-Arab, Alexandria and returned to the laboratory maintained prior to experimentation, where they were placed in separate large tanks (10 L) and/or bucket each with filtered tap water. The different larvae

Fig. 2 A photo of the studied animals *Bufo viridis* and *Bufo regularis* in their adult form. On the left is the green toad *Bufo viridis* with the orange spots, and on the right is the Egyptian toad *Bufo regularis* with the brown skin and green spots.

Fig. 3 A photo of tadpoles of the two species under study, *Bufo viridis* and *Bufo regularis*. On the left is the green toad *B. viridis* with the light green colour, and on the right is the Egyptian toad *B. regularis* with the dark brown colour.
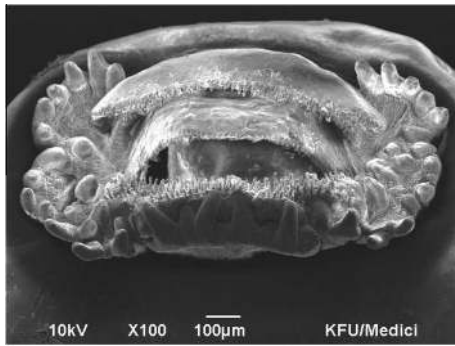

Fig. 4a A photo of oral disc of tadpoles of *Bufo viridis* showing the labial teeth rows. Note: two rows in upper labium and three in lower labium.

groups and their replicates, according to the following experimental design were placed in separate plastic pens $22 \times 16 \times 10.5$ cm with a suitable amount of dechlorinated tap water. *B. viridis* tadpoles were divided into two groups (Group A and Group B) that were randomly collected from natural ponds in Bourg El-Arab region, in the duration from April, 2009 to the end of June, 2009 and raised in

lab; other *B. viridis* tadpoles raised and examined in natural ponds as a third group, control group (Group C) were included.

Group A composed of eight treatments (AI, AII, AIII, AIV, AV, AVI, AVII, AVIII) that all tadpoles are raised in deep water pans and the other three factors are manipulated at different levels.

Group B composed of eight treatments (BI, BII, BIII, BIV, BV, BVI, BVII, BVIII) that all tadpoles are raised in shallow water pans and the other three factors are manipulated at different levels.

Fig. 4b A photo micrograph of oral disc of tadpoles of *Bufo viridis* showing the Labial Teeth Rows Formula (LTRF). Note: two rows in upper labium and three in lower labium.

Group C composed of tadpoles from natural ponds.

Performance of *B. viridis* varied widely among natural and experimental ponds with survivorship to metamorphosis, metamorphic size, metamorphic mass, and larval period each contributing to variation among ponds. The developmental duration in the observed natural ponds depended, in part, on pond duration and the variation in pond duration primarily depended on initial depth. In natural ponds, tadpoles were able to metamorphose with a snout-vent length range 12.40 ± 0.49 mm, mass range of 1.06 ± 0.1 and toadlets needed 40–43 days to emerge. The means and standard deviations of these variables are represented in Tables 1–5. To provide habitat structure each tank contained thin layers of field soil. Water was renewed twice a week. Rations of finely chopped spinach were weighed to 0.1 g on a Sartorious A210P balance and added daily. We evaluated variation of external morphology in tadpoles and toadlets from the experimental ponds. The following morphological traits were measured in tadpoles and toadlets: snout-to-vent length (SVL), fore limb length (FL), hind limb length (HLL), body length (BL), and only in tadpoles, tail length (TL) (Fig. 5). We included limb measurements because it has been documented that the hormones that control metamorphosis also control limb development in anurans (Emerson, 1986; Wassersug and Hoff, 1982).

Additionally, Lutz and Rome (1994) suggested that some larval environments, such as high larval density, induce allometry in the muscular structure of the hind limbs. So there is evidence to suspect that the relative limb sizes at metamorphosis are sensitive to development time as a function of desiccation risk. Measurements were made using a dissecting microscope incorporating an ocular micrometre (0.1 mm precision). Animals were narcotized by using MS 222 (amphibian anaesthetic, Sandoz A.G., Basel) in tap water at the concentration of 1:4000 and then measured to the nearest 0.55 mm snout-tail tip. After recovery from anaesthesia tadpoles were returned to their tanks. Metamorphosing individuals in different experiments were collected from the tanks as soon as fore limbs appeared. These were transferred to separate plastic boxes lined with damp tissue paper to complete metamorphosis. When the tail fully disappeared, at stage 66 (Sedra and Michael, 1961), the snout-vent length was measured and the metamorphs were removed. The food ration was adjusted for the remaining tadpoles in a box (for medium and high density treatments) to preserve a constant per capita ration.

Statistical analysis

Statistical tests were performed using STATISTICA, Version 5.0, Statosoft (1999). For all the life history traits we used tank mean values to ensure independent data. The following analyses were performed:

Descriptive statistics

This includes the mean values and standard deviations done at intervals of 4 days among the different 16 treatments according to Clark (1993).

Linear correlation

This is used to test the relationship between larval period, mass, and survival on one end and the different factors as pond duration, food level, density, and competition on the other end. The relationship between time and developmental stage following Sedra and Michael (1961) was well defined by a linear regression line (developmental stages as a function of time): $Y = a + b * x$.
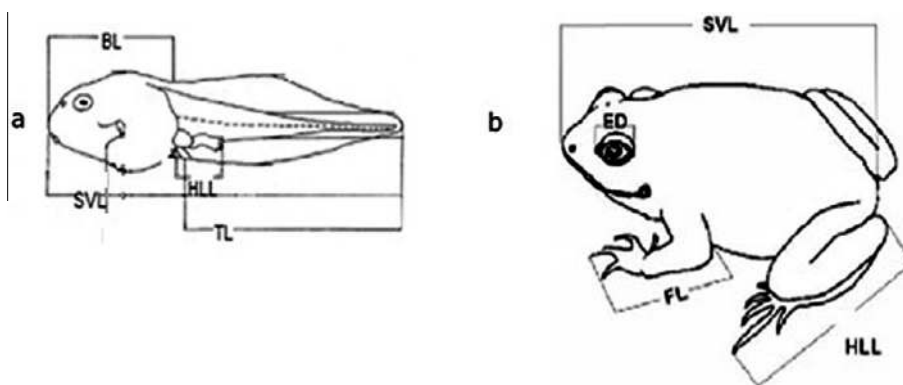
Fig. 5 Schematizations of the landmarks used for morphometric measurements in (a) tadpoles and (b) toadlets of *B. viridis*. The measured traits were: SVL = snout-to-vent length; FL = fore limb length; HLL = hind limb length; BL = body length; and TL = tail length.

Table 1 Univariate ANOVAS for mean snout-vent length.

Source	Mean ± SD
AI – Deep water + High food + Low density + No competition	27.85 ± 9.37
AII – Deep water + High food + High density + No competition	27.14 ± 8.76
AIII – Deep water + High food + Low density + Competition	27.61 ± 8.78
AIV – Deep water + High food + High density + Competition	26.33 ± 8.50
AV – Deep water + Low food + Low density + No competition	24.42 ± 8.48
AVI – Deep water + Low food + High density + No competition	22.57 ± 8.70
AVII – Deep water + Low food + Low density + Competition	21.76 ± 8.37
AVIII – Deep water + Low food + High density + Competition	20.46 ± 8.54
Control	20.97 ± 8.54
F (p)	14.53[*] (0.017)
LSD 5%	1.79
Source	Mean ± SD
BI – Shallow water + High food + Low density + No competition	11.72 ± 7.06
BII – Shallow water + High food + High density + No competition	20.77 ± 7.68
BIII – Shallow water + High food + Low density + Competition	20.89 ± 7.72
BIV – Shallow water + High food + High density + Competition	19.14 ± 7.23
BV – Shallow water + Low food + Low density + No competition	17.34 ± 6.80
BVI – Shallow water + Low food + High density + No competition	15.58 ± 7.20
BVII – Shallow water + Low food + Low density + Competition	14.73 ± 7.14
BVIII – Shallow water + Low food + High density + Competition	13.50 ± 7.57
Control	20.97 ± 8.45
F (p)	12.82[*] (<0.001)
LSD 5%	1.74

F: F test f (ANOVA).
(LSD): least significant difference.
  [*] Statistically significant at $p \leqslant 0.05$.

Table 2 Univariate ANOVAS for mean body length.

Source	Mean ± SD
AI – Deep water + High food + Low density + No competition	16.96 ± 4.85
AII – Deep water + High food + High density + No competition	16.25 ± 7.93
AIII – Deep water + High food + Low density + Competition	15.84 ± 4.58
AIV – Deep water + High food + High density + Competition	15.36 ± 4.33
AV – Deep water + Low food + Low density + No competition	14.06 ± 4.50
AVI – Deep water + Low food + High density + No competition	13.47 ± 4.44
AVII – Deep water + Low food + Low density + Competition	13.31 ± 7.25
AVIII – Deep water + Low food + High density + Competition	12.93 ± 4.13
Control	12.16 ± 3.99
F (p)	32.18[*] (0.115)
LSD 5%	1.211
Source	Mean ± SD
BI – Shallow water + High food + Low density + No competition	8.72 ± 2.02
BII – Shallow water + High food + High density + No competition	12.78 ± 4.22
BIII – Shallow water + High food + Low density + Competition	12.38 ± 3.36
BIV – Shallow water + High food + High density + Competition	10.92 ± 3.12
BV – Shallow water + Low food + Low density + No competition	11.73 ± 3.40
BVI – Shallow water + Low food + High density + No competition	9.99 ± 2.39
BVII – Shallow water + Low food + Low density + Competition	9.16 ± 2.06
BVIII – Shallow water + Low food + High density + Competition	8.22 ± 2.01
Control	12.61 ± 3.99
F (p)	28.409[*] (0.001)
LSD 5%	0.975

F: F test f (ANOVA).
  [*] Statistically significant at $p \leqslant 0.05$.

Table 3 Univariate ANOVAS for mean tail length.

Source	Mean ± SD
AI – Deep water + High food + Low density + No competition	16.96 ± 4.85
AII – Deep water + High food + High density + No competition	16.25 ± 7.93
AIII – Deep water + High food + Low density + Competition	15.84 ± 4.58
AIV – Deep water + High food + High density + Competition	15.36 ± 4.33
AV – Deep water + Low food + Low density + No competition	14.06 ± 4.50
AVI – Deep water + Low food + High density + No competition	13.47 ± 4.44
AVII – Deep water + Low food + Low density + Competition	13.31 ± 7.25
AVIII – Deep water + Low food + High density + Competition	12.93 ± 4.13
Control	12.16 ± 3.99
F (p)	32.18[*] (0.115)
LSD 5%	1.211
Source	Mean ± SD
BI – Shallow water + High food + Low density + No competition	8.72 ± 2.02
BII – Shallow water + High food + High density + No competition	12.78 ± 4.22
BIII – Shallow water + High food + Low density + Competition	12.38 ± 3.36
BIV – Shallow water + High food + High density + Competition	10.92 ± 3.12
BV – Shallow water + Low food + Low density + No competition	11.73 ± 3.40
BVI – Shallow water + Low food + High density + No competition	9.99 ± 2.39
BVII – Shallow water + Low food + Low density + Competition	9.16 ± 2.06
BVIII – Shallow water + Low food + High density + Competition	8.22 ± 2.01
Control	12.61 ± 3.99
F (p)	28.409[*] (0.001)
LSD 5%	0.975

F: F test f (ANOVA).
[*] Statistically significant at $p \leqslant 0.05$.

ANOVA test

Analysis of variance of body length of metamorphs and length of larval period together with pond duration were performed by univariate ANOVA according to Sokal and Rohlf (1981). The effects of competition, food level, and pond duration and interactions between them on life history traits (larval period, mass at metamorphosis, growth rate, survival to stage 66, and mortality rate between forelimb emergence and tail resorption). If a significant changes were indicated, levels of significance were inferred at $P < 0.01$.

Result

Body size and growth

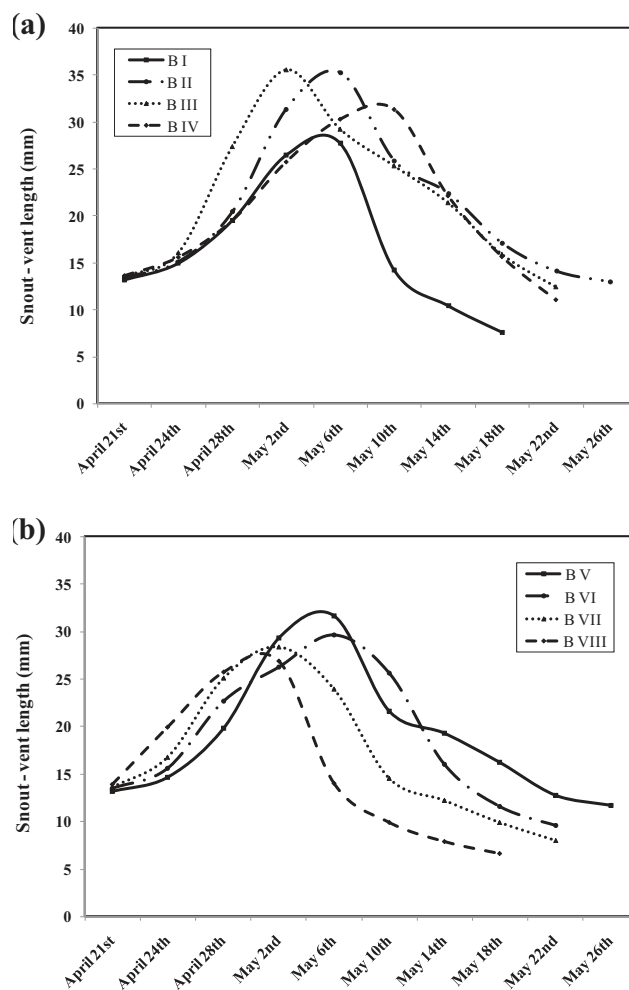##### Snout-vent, body and tail lengths

The snout-vent length increased in a linear pattern from developmental stage 42 until stage 57/58, after which larvae began to shorten their tail. Analysis of variance test between the deep water and shallow water pens was performed showing a significant difference between the two pens for snout-vent length (deep water: $F = 14.53$, $P = 0.017$, shallow water: $F = 12.82$, $P < 0.001$, Table 1), body length (deep water: $F = 32.18$, $P = 0.115$, shallow water: $F = 28.409$, $P = 0.001$, Table 2) and tail length (deep water: $F = 11.91$, $P = 0.003$, shallow water: $F = 5.537$, $P < 0.001$, Table 3). Tadpoles responded to desiccation by accelerating growth. This acceleration lead to significant differences in SV, body and tail lengths during metamorphosis. Tadpoles of deep water pens (AI, AII, AIII and AIV) reached the largest snout-vent length that ranged between 41.22 and 33.38 mm at stage 57/58 and 18.29 and 13.35 mm at metamorphosis (Fig. 6a and b), body length ranged between 18.16 and 13.35 mm at stage 64/65 after which it remained stationary (Fig. 7a and b) and longest tail length ranged between 23.67 and 20.44 mm at stage 57/58 after which it began to shorten until it is all resorbed (Fig. 10a and b). In shallow water pens (BI, BII, BIII and BIV) tadpoles reached the smallest snout-vent length that ranged between 33.85 and 25.06 mm at stage 57/58 and 11.58 and 6.84 mm at metamorphosis (Fig. 8a and b), body length ranged between 11.59 and 6.31 mm at stage 64/65 (Fig. 9a and b) and the shortest tail length ranged between 18.63 and 14.18 mm at stage 57/58 after which it began to shorten until it is all resorbed (Fig. 11a and b). An ANOVA test showed that overall size trajectories varied significantly among high and low food amount treatments. Tadpoles provided with high food in deep water pens (AI, AII, AIII and AIV) metamorphosed at the largest sizes. Significant differences were apparent as a result of small sizes in the low food pens, tadpoles metamorphosed at small sizes in deep water pens (AV, AVI, AVII and AVIII), smaller in high food and shallow water pens (BI, BII, BIII and BIV) and reached the smallest size at low food and shallow water pens (BV, BVI, BVII and BVIII). This shows that both water level and food amount have the same effect on SV, body and tail lengths. Tadpoles in deep water pens with high density or intraspecific competition (AII, AIV, AVI and AVIII) were significantly different and smaller in size than those in deep water pens and low density (AI, AIII, AV and AVII). For shallow water pens where tadpoles under high interspecific competition (BII, BIV, BVI and BVIII)
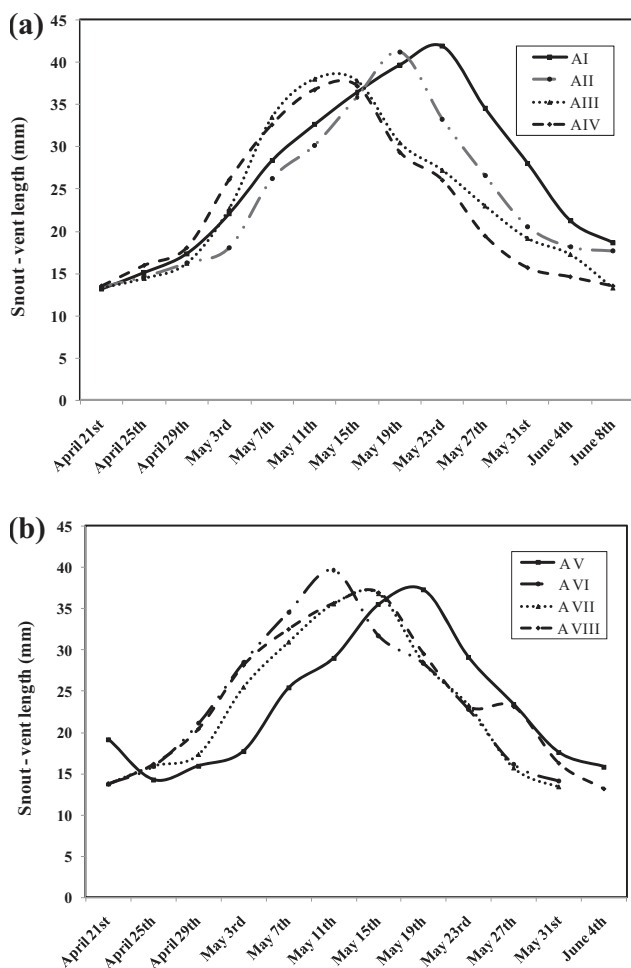
Fig. 6 Relationship between time (days) and larval snout-vent length (mm) in the deep water pens (A). (a) Deep water and high food pens (AI–AIV). (b) Deep water and low food amount pens (AV–AVIII). Day 1 = April 21st at which the experiment started. Due to overlapping, each dot represents means of numerous tadpoles.

Fig. 7 Relationship between time (days) and larval snout-vent length (mm) in the shallow water pens (B). (a) Shallow water and high food pens (BI–BIV). (b) Shallow water and low food amount pens (BV–BVIII). Day 1 = April 21st at which the experiment started. Due to overlapping, each dot represents means of numerous tadpoles.

tadpoles were significantly smaller in size than those in pens with low interspecific competition (BI, BIII, BV and BVII). The presence of *B. regularis* as interspecific competition in deep pens (AII, AIV, AVII and AVIII) and in shallow pens (BII, BIV, BVII and BVIII) had a significant effect on sizes of tadpoles and leads to smaller size than those in deep and shallow pens without interspecific competition (AI, AIII, AV, AVI, BI, BIII, BV and BVI). It was noticed that tadpoles in pens are common in all conditions except food level (AII vs. AVII, AIII vs. AVIII, BII vs. BVII and BIII vs. BVIII). All these pens are common in intra- and interspecific competitions but differ in food levels. This indicates that food amount has a more significant effect than competition on SV, body and tail lengths during development. Among all the treatments, pens AVIII, BII and BIII were the only pens of insignificant differences with the control group. BI was significant with all the pens and control. The hind limbs start to emerge as tiny limb buds at stage 50/51 and continues to grow until the tail degenerates during metamorphosis. Larvae of

the deep water pens reached hind limb length that ranged between 13.06 and 10.91 mm at metamorphosis (Fig. 12a and b) while larvae of the shallow water pens reached hind limb length that ranged between 10.22 and 7.59 mm at metamorphosis (Fig. 13a and b). The fore limbs start to project through the operculum, left side first at stage 52/53 then the right side at stage 53/54 and continue to grow until metamorphosis. Larvae of the deep water pens reached fore limb length that ranged between 8.55 and 6.61 mm at metamorphosis (Fig. 14a and b). Larvae of the shallow water pens reached fore limb length that ranged between 3.24 and 5.51 mm at metamorphosis (Fig. 15a and b). ANOVA test for both hind and fore limbs lengths showed there were significant differences between the deep water (A) and the shallow water (B) pens and different food levels but there was no significant effect of intra- or interspecific competition. For hind limb deep water $F = 2.303$, $P = 0.019$, LSD = 1.207; shallow water $F = 4.618$, $P < 0.001$, LSD = 1.967; Table 4; while fore limb deep water $F = 2.533$, $P = 0.010$, LSD = 0.772; shallow water $F = 6.606$, $P < 0.001$, LSD = 0.618; Table 5.
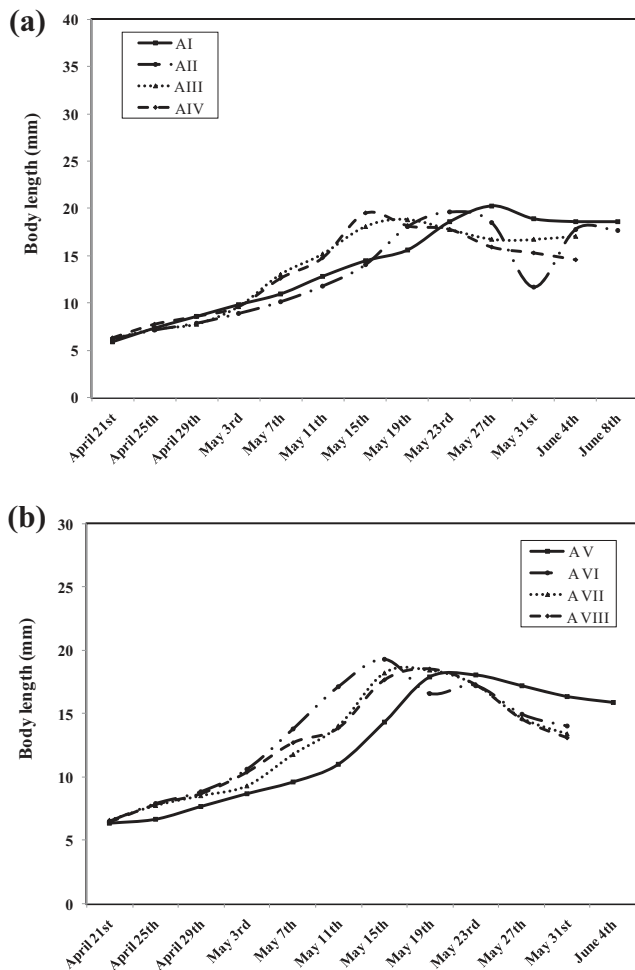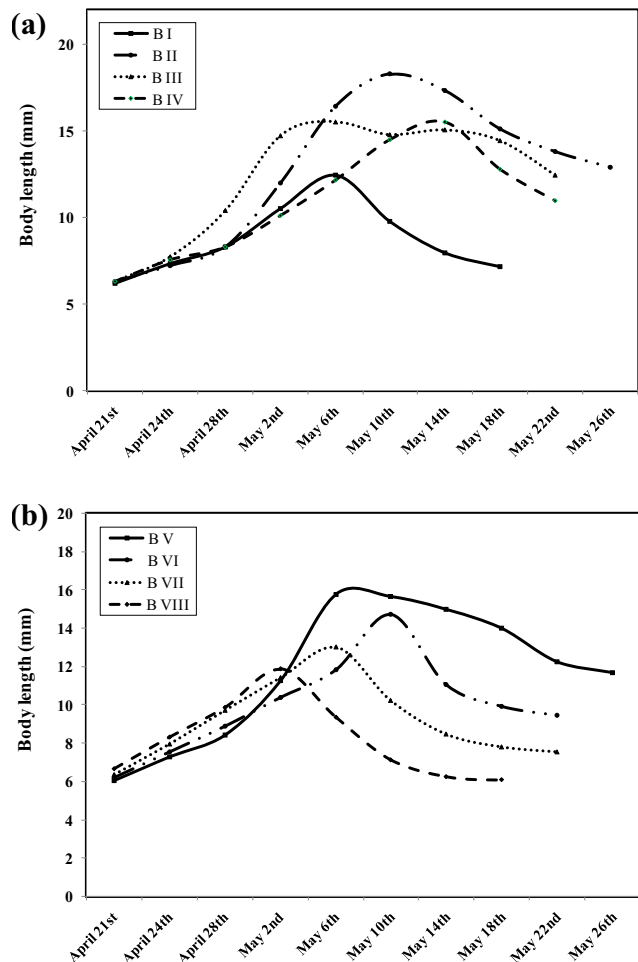
Fig. 8 Relationship between time (days) and larval body length (mm) in the deep water pens (A). (a) Deep water and high food pens (AI–AIV). (b) Deep water and low food amount pens (AV–AVIII). Day 1 = April 21st at which the experiment started. Due to overlapping, each dot represents means of numerous tadpoles.

Fig. 9 Relationship between time (days) and larval body length (mm) in the shallow water pens (B). (a) Shallow water and high food pens (BI–BIV). (b) Shallow water and low food amount pens (BV–BVIII). Day 1 = April 21st at which the experiment started. Due to overlapping, each dot represents means of numerous tadpoles.

Survivorship

Pond duration significantly affects larval survival prior to pond drying. Larval survival in deep water and high food treatments was high (experiments AI, AII, AIII and AIV), averaging 95%, 94.4%, 85% and 84.9% respectively, but survived slightly less well in deep water and low food amount ponds (experiments AV, AVI, AVII, and AVIII), averaging 82.5%, 83.3%, 75% and 80% respectively. In the shallow water and high food treatments, the survival rate was still relatively high (experiments BII, BIII and BIV), averaging 74.4%, 67.5% and 65.4% respectively; the exception was 30% at the first pond BI which was the least among all the treatments.

In the remaining four treatments of shallow water and low food (experiments BV, BVI, BVII and BVIII), the survival was low averaging 60%, 61.7%, 40% and 45.1% respectively. The percentage surviving is shown in relation to time (in days) in Fig. 16.

Discussion

Several studies illustrated that when abiotic and biotic conditions provide performance benefits during the larval stage, metamorphic size is often larger (Merilä et al., 2004; Lardner, 2000; Van Buskirk, 2000; Doughty and Roberts, 2003). According to Waringer-Loschenkohl et al. (2003), in natural ponds it is a distinct ecological advantage to leave the water as early as possible in order to escape the high predation pressure and the ever increasing desiccation risk in the summer season; however, a shorter larval period often means smaller toadlets at metamorphosis. Richter-Boix et al. (2006) evaluated the effect of pond desiccation on life-history traits and the morphology of tadpoles and toadlets in *Pelodytes punctatus*. They found that tadpoles subjected to a drying treatment accelerated metamorphosis and reached this stage with a lower body mass. Larvae in more-permanent

(a)

(b)
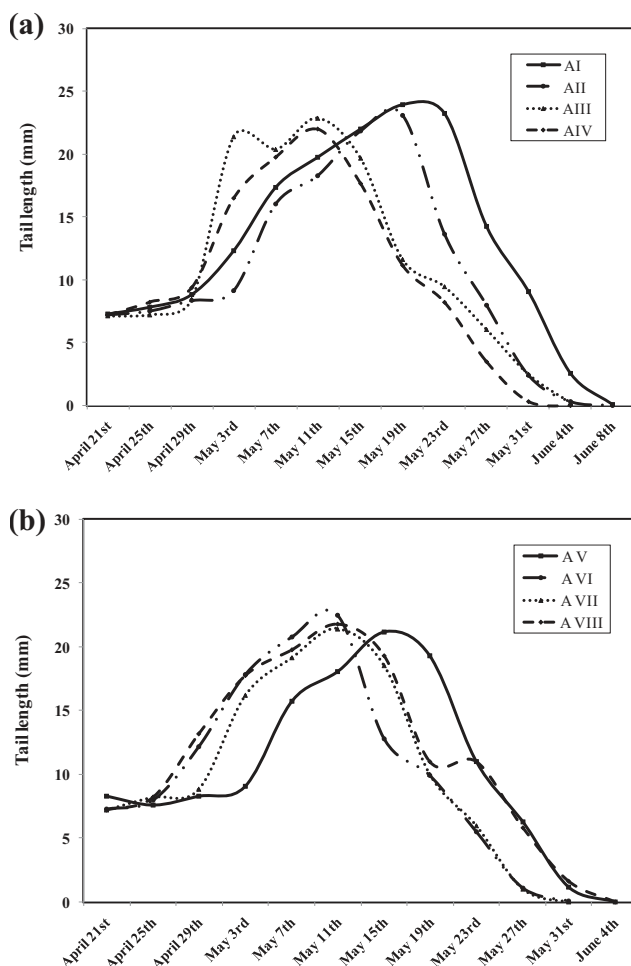
Fig. 10 Relationship between time (days) and larval tail length (mm) in deep water pens (A). (a) Deep water and high food pens (AI–AIV). (b) Deep water and low food amount pens (AV–AVIII). Day 1 = April 21st at which the experiment started. Due to overlapping, each dot represents means of numerous tadpoles.
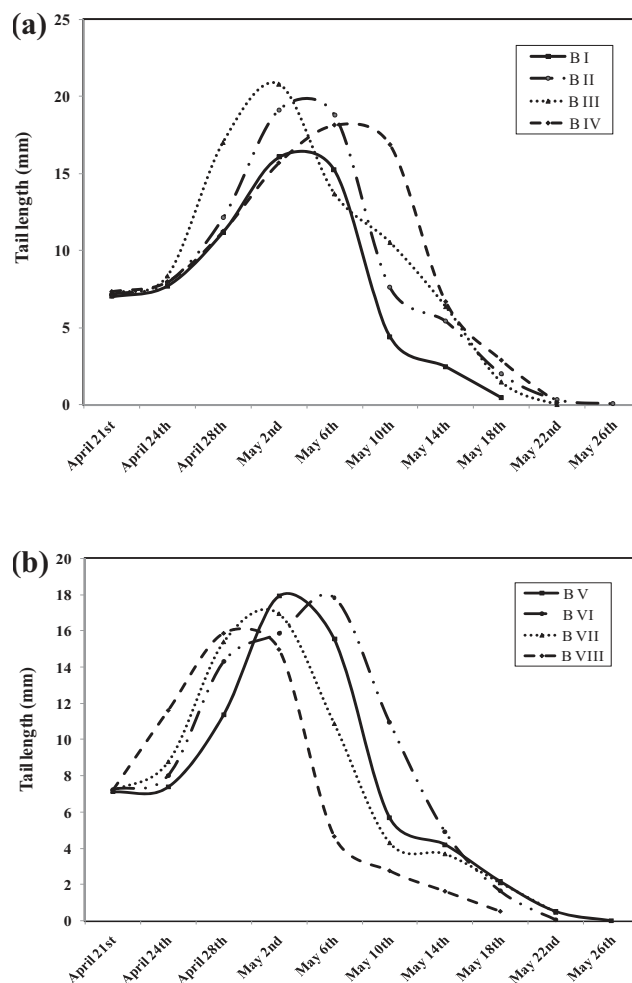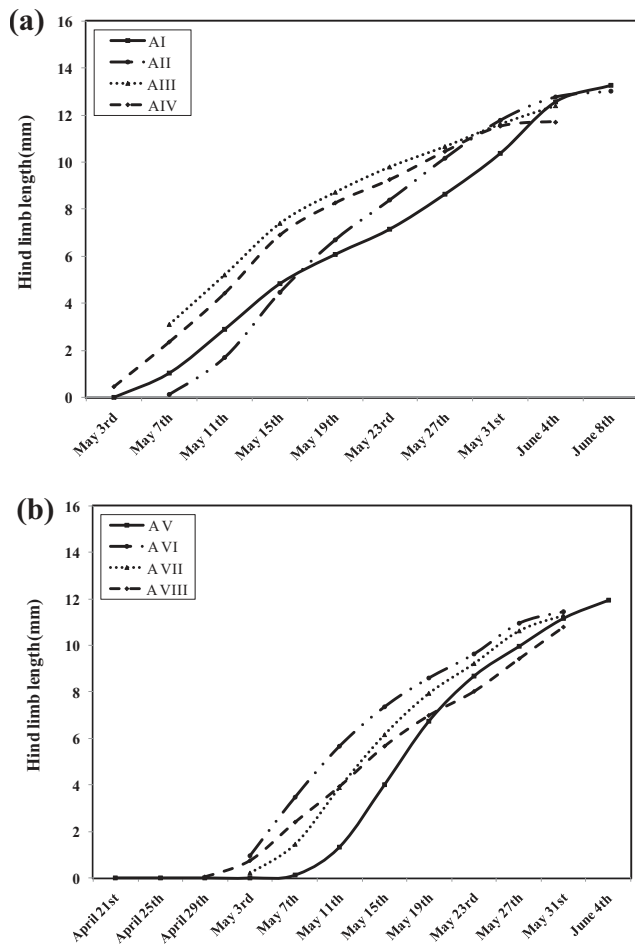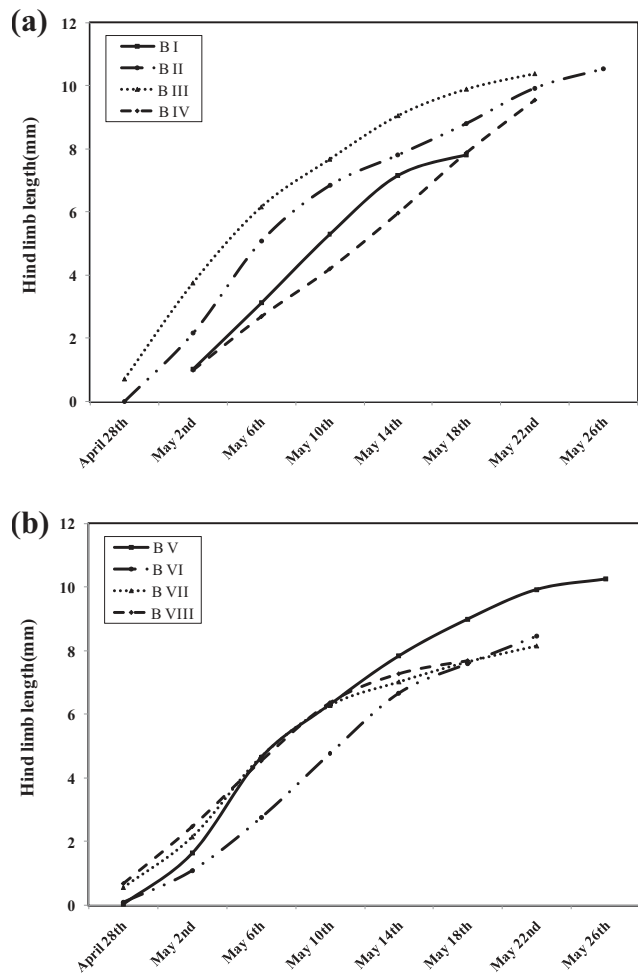
(a)

(b)

Fig. 11 Relationship between time (days) and larval tail length (mm) in the shallow water pens (B). (a) Shallow water and high food pens (BI–BIV). (b) Shallow water and low food amount pens (BV–BVIII). Day 1 = April 21st at which the experiment started. Due to overlapping, each dot represents means of numerous tadpoles.

environments reach larger sizes than larvae in ponds with brief hydroperiods (Székely et al., 2010; Degani et al., 2012). Other studies of amphibian development have also demonstrated smaller size at metamorphosis from desiccating ponds but, in contrast to the present study, this has been accompanied by accelerated development (Wilbur, 1987; Newman, 1989; Griffiths and Brady, 2000). Such results can be interpreted in terms of the model described by Smith-Gill and Berven (1979) which suggests that growth and development are uncoupled in tadpole development. Also, Griffiths and Brady (2000) stated that amphibians metamorphosed at a smaller size from desiccating ponds than from non-desiccating ponds, but the timing of metamorphosis was unaffected. They explained their results by a single programme of development being expressed in different habitat types while under physiological constraints. Other studies based on natural environments (Degani, 1986) and laboratory condition (Degani, 1993) results were also not compatible with this study. They stated that metamorphic size is often larger and larval duration is commonly shorter.

Opposing the present results, Reques and Tejedo (1997) found that there was no plastic response to pond duration. Similarly, Gervasi and Foufopoulos (2008), noticed the main effect of desiccation in their experiment was on development or differentiation rate, not growth rate. According to Degani et al. (2012) no significant differences were observed when comparing the age at metamorphosis between tadpoles of *B. viridis* in five ephemeral aquatic breeding sites. Accordingly, desiccation influenced age but not size or mass at metamorphosis and this is not compatible with the current results. They considered the variations in age at metamorphosis of tadpoles among individuals of the same species in different habitats to allow species to complete metamorphosis in habitats with extreme conditions, occupy more habitats and become more widely distributed. In terms of possible allometric changes associated with desiccation, the allometric relationship between shape and size is not constant, but rather is a function of development rate (Blouin and Loeb, 1991). Thus, environment might induce morphometric variation simply by controlling
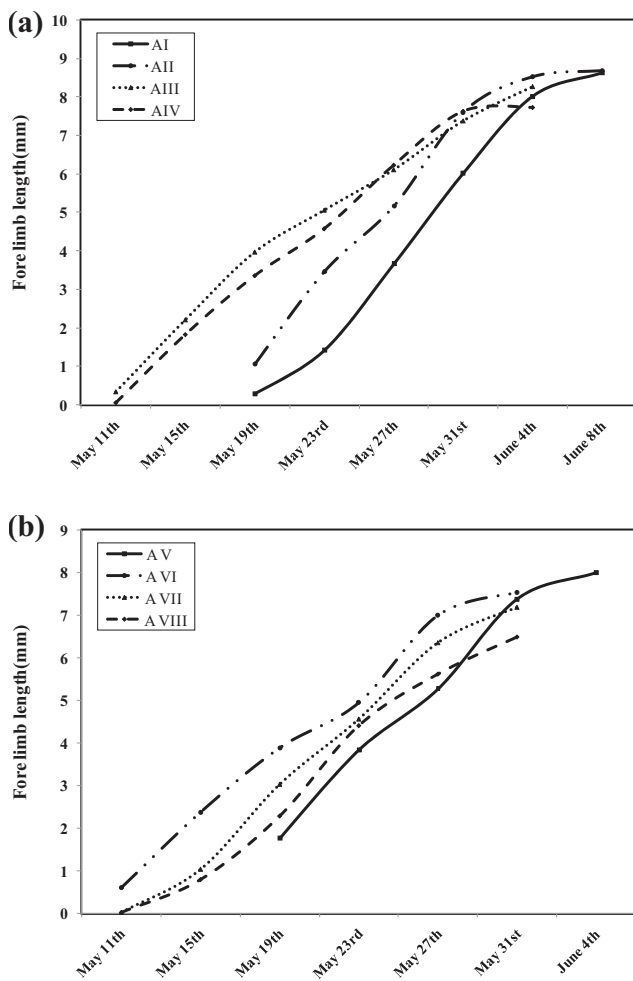
Fig. 12 Relationship between time (days) and larval hind limb length (mm) in the deep water pens (A). (a) Deep water and high food pens (AI–AIV). (b) Deep water and low food amount pens (AV–AVIII). Day 1 = April 21st at which the experiment started. Due to overlapping, each dot represents means of numerous tadpoles.

Fig. 13 Relationship between time (days) and larval hind limb length (mm) in the shallow water pens (B). (a) Shallow water and high food pens (BI–BIV). (b) Shallow water and low food amount pens (BV–BVIII). Day 1 = April 21st at which the experiment started. Due to overlapping, each dot represents means of numerous tadpoles.

the overall rates of growth and differentiation (Blouin and Brown, 2000). It has been documented that leg length is a functionally important trait in anurans and that differences in growth rate may produce changes in this character (Emerson, 1986; Newman, 1994; Blouin and Brown, 2000). The current results also show longer fore and hind limbs in deep water pens (low desiccation rate), apparently related to the extension of larval period. It was also noticed that relative hindlimb length has been shown to be positively correlated with growth rate and negatively correlated with time to metamorphosis. Blouin and Brown (2000) described a similar pattern of variation in *Rana cascadae*. In their study, tadpoles with a longer larval period had a longer tibia-to-fibula length, showing plasticity in this trait independent of body size. Growth rate may limit metabolic scope, reducing the energy available for locomotion (Conover and Schultz, 1997; Billerbeck et al., 2001). Other studies have also reported shorter hind limbs under pond desiccation risk, e.g. in *Scaphiopus couchii* (Newman, 1989) and *Rhinella spinulosa*
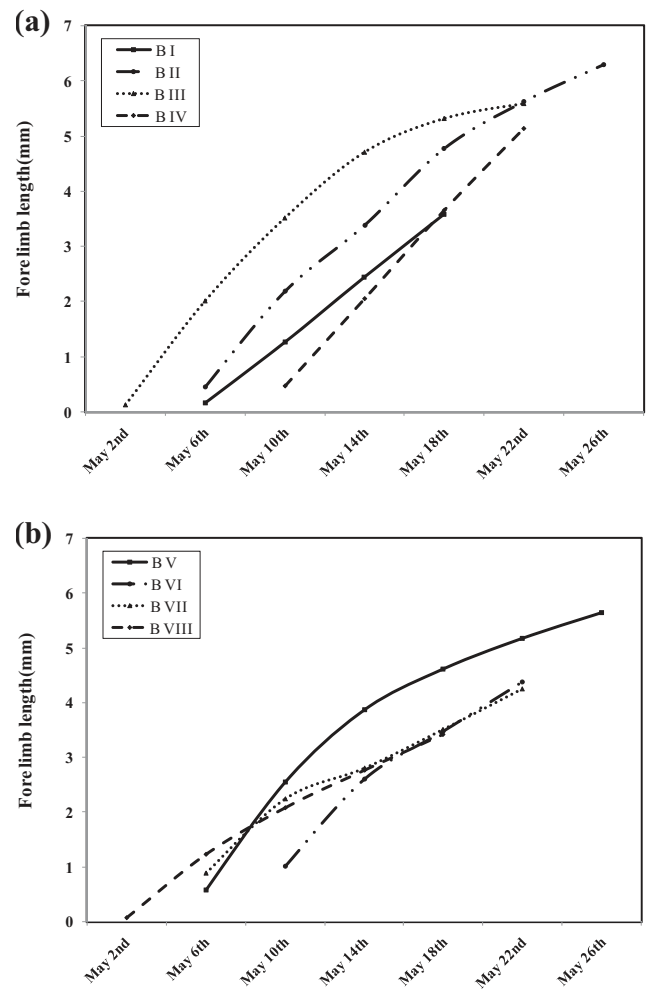
(Márquez-García et al., 2009). Relyea and Hoverman (2003) found that fore limb width and body width were not significantly affected by the plastic effects of larval competition until a month after metamorphosis. Méndez et al. (2009) showed that desiccation had an effect on hind limb length. These morphological traits varied independently of body size. Tejedo et al. (2010) illustrated that stressful conditions operating during larval stages (lower resource levels or desiccation risk) seem to promote a faster whole-body developmental rate that allows larvae to escape from a poor growing environment at the cost of incomplete development of some body parts (e.g. hind limbs). Plasticity of hind limb length was of relatively low or moderate magnitude, especially if compared with plasticity in development, growth rate or mass at metamorphosis. Interspecific comparisons have suggested that an increase in leg length is needed to have a substantial influence on locomotion. The survival rate was very high among the deep tanks and low among the shallow tanks. The relatively high survival of *B. viridis* in shallow water pens, may be due to the early

(a)

(b)

Fig. 14 Relationship between time (days) and larval fore limb length (mm) in the deep water pens (A). (a) Deep water and high food pens (AI–AIV). (b) Deep water and low food amount pens (AV–AVIII). Day 1 = April 21st at which the experiment started. Due to overlapping, each dot represents means of numerous tadpoles.

(a)

(b)

Fig. 15 Relationship between time (days) and larval fore limb length (mm) in shallow water pens (B). (a) Shallow water and high food pens (BI–BIV). (b) Shallow water and low food amount pens (BV–BVIII). Day 1 = April 21st at which the experiment started. Due to overlapping, each dot represents means of numerous tadpoles.

metamorphosis and therefore managed to escape desiccation. This agrees with results of Márquez-García et al. (2009) on *R. spinulosa*. The experimental manipulations in this study prove that interactions with desiccation risk and food resource availability seem to be stronger than intra- and interspecific competition. Also it appears that the environmental heterogeneity promotes phenotypic variation in both morphology and life-history traits of *B. viridis* tadpoles. One possibility is that these species have evolved traits that allow them to exploit transient habitats that are rich in food resources and low in competitors and predators.

Finally, documenting the recent results of this study, *B. viridis* breed in temporary ponds and exhibited plasticity in developmental duration and growth rate in response to a change in water level, and this response varied in direct relation to the magnitude of the environmental signal. Metamorphosis in response to pond drying may be as much an

adaptation to the temporary desert ponds as is the rapid development achieved by desert species. Pond duration significantly affects larval survival prior to pond drying. Larval survival in deep water and high food treatments was high (experiments AI, AII, AIII and AIV), averaging 95%, 94.4%, 85% and 84.9% respectively. They survived slightly less well in deep water and low food amount ponds (experiments AV, AVI, AVII, and AVIII), averaging 82.5%, 83.3%, 75% and 80% respectively. In shallow water and high food treatments, the survival rate was still relatively high (experiments BII, BIII and BIV), averaging 74.4%, 67.5% and 65.4% respectively. The exception was 30% at the first pond BI which was the least among all the treatments. In the remaining four treatments of shallow water and low food (experiments BV, BVI, BVII and BVIII), the survival was low averaging 60%, 61.7%, 40% and 45.1% respectively. The percentage surviving is shown in relation to time (in days) in Fig. 15.

Table 4 Univariate ANOVAS for mean hind limb length.

Source	Mean ± SD
AI – Deep water + High food + Low density + No competition	5.14 ± 4.78
AII – Deep water + High food + High density + No competition	5.01 ± 5.05
AIII – Deep water + High food + Low density + Competition	5.74 ± 4.78
AIV – Deep water + High food + High density + Competition	5.17 ± 4.51
AV – Deep water + Low food + Low density + No competition	4.17 ± 4.55
AVI – Deep water + Low food + High density + No competition	5.28 ± 4.42
AVII – Deep water + Low food + Low density + Competition	4.30 ± 4.25
AVIII – Deep water + Low food + High density + Competition	4.06 ± 3.71
Control	3.99 ± 3.67
$F(p)$	2.303[*] (0.019)
LSD 5%	1.207
Source	Mean ± SD
BI – Shallow water + High food + Low density + No competition	3.06 ± 3.14
BII – Shallow water + High food + High density + No competition	5.12 ± 4.10
BIII – Shallow water + High food + Low density + Competition	5.30 ± 4.09
BIV – Shallow water + High food + High density + Competition	3.47 ± 3.49
BV – Shallow water + Low food + Low density + No competition	4.97 ± 4.09
BVI – Shallow water + Low food + High density + No competition	3.49 ± 3.29
BVII – Shallow water + Low food + Low density + Competition	4.05 ± 3.25
BVIII – Shallow water + Low food + High density + Competition	3.58 ± 3.08
Control	3.99 ± 3.67
$F(p)$	4.618[*] (<0.001)
LSD 5%	1.967

F: F test f (ANOVA).
[*] Statistically significant at p ⩽ 0.05.

Table 5 Univariate ANOVAS for mean forelimb length.

Source	Mean ± SD
AI – Deep water + High food + Low density + No competition	2.17 ± 3.21
AII – Deep water + High food + High density + No competition	2.41 ± 3.33
AIII – Deep water + High food + Low density + Competition	2.77 ± 3.10
AIV – Deep water + High food + High density + Competition	2.90 ± 2.91
AV – Deep water + Low food + Low density + No competition	1.93 ± 2.81
AVI – Deep water + Low food + High density + No competition	2.39 ± 2.86
AVII – Deep water + Low food + Low density + Competition	1.77 ± 2.51
AVIII – Deep water + Low food + High density + Competition	1.56 ± 2.26
Control	1.53 ± 2.24
$F(p)$	2.533[*] (0.010)
LSD 5%	0.772
Source	Mean ± SD
BI – Shallow water + High food + Low density + No competition	0.93 ± 1.32
BII – Shallow water + High food + High density + No competition	2.27 ± 2.45
BIII – Shallow water + High food + Low density + Competition	2.39 ± 2.37
BIV – Shallow water + High food + High density + Competition	1.25 ± 1.87
BV – Shallow water + Low food + Low density + No competition	2.21 ± 2.28
BVI – Shallow water + Low food + High density + No competition	1.27 ± 1.67
BVII – Shallow water + Low food + Low density + Competition	1.52 ± 1.64
BVIII – Shallow water + Low food + High density + Competition	1.67 ± 1.31
Control	1.52 ± 2.24
$F(p)$	6.606[*] (<0.001)
LSD 5%	0.618

F: F test f (ANOVA).
[*] Statistically significant at p ⩽ 0.05.
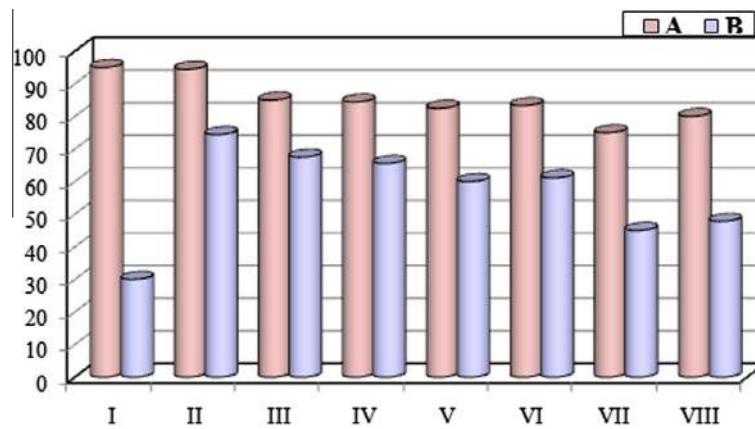
Fig. 16 Histogram representing larval survival in the 16 pens (at different levels of pond duration, food, intra- and interspecific competition).

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