

Phenotypic Plasticity in Larval Development of Six Amphibian Species in Stressful Natural Environments

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Tali Goldberg, Eviatar Nevo, and Gad Degani (2012) Phenotypic plasticity in larval development of six amphibian species in stressful natural environments. *Zoological Studies* 51(3): 345-361. Phenotypic plasticity is known as the capacity to change in response to different environmental conditions, and if these changes imply reversible transformations, it is known as phenotypic flexibility. This plasticity includes changes in behavior, physiology, morphology, growth, life history, and demography, and can be expressed either within the lifespan of a single individual or across generations. 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 favorable. Plasticity may allow tadpoles to avoid mortality in a desiccating habitat by accelerating metamorphosis and reducing their size at metamorphosis. This study examined 6 species of amphibian larvae over several years that grew and completed metamorphosis at 14 ephemeral and permanent breeding sites in a Mediterranean climate. The aim of the current study was to test the ability of these 6 species to undergo phenotypic changes in larval size and the course of metamorphosis relative to time, in response to various water-quality parameters. Of the 6 species tested, the salamander was the only species that occupied all types of breeding sites that were sampled and showed significant differences in growth rates and sizes of tadpoles during metamorphosis by comparison between individuals from different pools. Five other species mainly inhabited ephemeral pools. There was no significant difference in the timing of metamorphosis, but for some species there was a significant difference in the final size of the tadpoles. In 2 poorly fed, completely dark, and very shallow breeding sites, we found *Salamandra infraimmaculata* larvae for longer periods and with a smaller size at metamorphosis. <http://zoolstud.sinica.edu.tw/Journals/51.3/345.pdf>

Key words: Amphibians, Ephemeral habitat, Permanent habitat, Larval growth.

Evolutionary biologists often use phenotypic differences between species and between individuals to gain an understanding of organismal design. Populations differ in (1) their phenotypic plasticity, which is the ability of an organism with a given genotype to change its phenotype in response to abiotic and biotic changes in its habitat (Price et al. 2003), or in (2) their phenotypic flexibility, reversible within-individual variation, which is a function of environmental conditions that vary predictably (e.g., by seasons) or stochastically

(Piersma and Drent 2003, Miner et al. 2005).

Amphibian larvae exhibit extreme phenotypic flexibility in life-history traits in response to their environment and trade-offs due to conflicting selection pressures, which may be particularly important in ectotherms with complex life cycles (Rot-Nikcevic et al. 2005). The aquatic phase of an amphibian population is a critical period in the life of many of its species. Amphibians breed in different habitats, including ephemeral ponds (e.g., rain pools), semi-permanent ponds (e.g., rock

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pool holes), and permanent aquatic habitats (e.g., springs and streams) (Degani and Kaplan 1999). Mediterranean aquatic habitats are frequently temporary, with short periods of available surface water from autumn to spring, a long summer drought, and highly variable hydrological conditions among years. Such conditions have significant impacts on the life cycle of amphibians (Jakob et al. 2002).

In unpredictable environments, a capacity for rapid and reversible phenotypic change (flexibility) has obvious fitness payoffs (Padilla and Adolph 1996). Where environmental conditions vary in a predictable temporary way, long-lived organisms can anticipate such changes by showing sequences of life-cycle stages (Jacobs and Wingfield 2000, Piersma 2002). The seasonal template for such sequences might be provided by the natural photoperiodic rhythm and/or by an endogenous circannual pacemaker (Gwinner 1986). In addition, temperature, rainfall, food, or densities of conspecifics might give supplementary information, which individuals can use to 'fine-tune' the timing of their phenotypic transformations.

Two of the most flexible traits of amphibians, timing of and size at metamorphosis, are both influenced by various abiotic and biotic factors (Scott 1990, Hayes et al. 1993, McCollum and Van Buskirk 1996). Changes in habitat, such as pond desiccation, limited food resources, and the presence of predators or competition, can all affect the duration of the larval stage and the timing of metamorphosis (Newman 1994, Denver et al. 2002). Size at metamorphosis is related to adult size (Werner 1986), and adult size is positively correlated with reproductive success (Altwegg and Reyer 2003).

A tadpole's growth rate is usually considered to be a result of a balance between selection for faster growth, which will decrease the high mortality risk (from predators and desiccation), and selection for slower growth, which demands less energy per unit of time and consequently allows for a larger ultimate size (Kehr 1998). Species inhabiting ephemeral sites have little time available for growth and development; thus, tadpoles that live in temporary wetlands tend to grow rapidly and try to reach metamorphosis before the water disappears (Woodward 1983, Wellborn et al. 1996). Thermoregulation is particularly important for these amphibians as the growth rate is closely related to temperature (Castañeda et al. 2006, Bancroft et al. 2008). In contrast, tadpoles from wetlands with long hydroperiods have slower growth rates

and tend to be less active than those in temporary wetlands (Gunzburger and Travis 2004). Species which inhabit more-permanent habitats tend to have defense strategies that enable them to coexist with potential predators (Woodward 1983, Góme and Kehr 2011). Moreover, there is thought to be a minimum size at which metamorphosis can occur (snout vent length or larval full length) (Wilbur and Collins 1973, Whiteman 1994, Day and Rowe 2002), and if larvae cannot grow fast enough to reach metamorphosis size before the environment becomes uninhabitable, they will perish before recruitment. There is thus an apparent trade-off between metamorphosis at large sizes (maximizing terrestrial survival and fecundity) and metamorphosis at small sizes (minimizing larval mortality) (Rudolf and Rödel 2007).

Variations in individual growth rates are demonstrated by large size variations observed in organisms from the same group reared together in controlled conditions or even in nearly equal conditions (Peacor and Pfister 2006). The coexistence of similar species in ecological communities, especially if they are closely related taxonomically and they exhibit overlapping activity periods, body sizes, and microhabitat features, often creates interactions with other organisms. The degree of overlap in the use of resources among co-occurring species varies and can affect growth rates (Bowker and Bowker 1979, Etges 1987, Menin et al. 2005).

Considering global warming, amphibians in Israel are an excellent model system to study phenotypic plasticity especially under the extreme conditions at the limits of their distributions. In a relatively small area, there are permanent water bodies and ephemeral habitats which are sporadically filled by rain, and in these habitats, tadpoles have a short development time. Plasticity in development time in species which breed in such ephemeral habitats allows an individual to prolong the larval period and maximize its size at metamorphosis when conditions are favorable. On the other hand, tadpoles can avoid mortality in a desiccating habitat by accelerating metamorphosis, thereby reducing size at metamorphosis.

The 6 amphibian species existing in northern Israel are 2 Urodela species, the striped newt *Triturus vittatus vittatus*, and the fire salamander *Salamandra infraimmaculata*, and 4 anurans, including the tree frog *Hyla savignyi*, green toad *Bufo viridis*, water frog *Rana bedriagae*, and spadefoot *Pelobates syriacus* (Degani 1982 1986). In a previous study on breeding places

of amphibian larvae, we mainly described the various water-quality parameters at breeding sites of temperature, pH, soluble oxygen (DO), conductivity, ammonium (NH_4^+), and invertebrate biomass, and tried to describe the ecological niches of larvae of these 6 amphibian species (Goldberg et al. 2009).

The aim of the current study was to explore the phenotypic flexibility of tadpoles of different species under natural conditions of various water-quality parameters of their habitats. Differences in growth rates of populations were investigated by examining amphibian full length during growth and at completion of metamorphosis in different types of breeding sites located in various habitats during several seasons. Results of this study should increase our understanding of larval development, growth, and complete metamorphosis in different water bodies. The data gathered herein are very important for nature conservation because the variety of species in Israel includes one listed as near threatened (*S. infraimmaculata*) and several species the populations of which are decreasing, as classified by the IUCN (2006).

MATERIALS AND METHODS

Study area

The study was carried out over 4 consecutive years (2005-2008), except for at sites Po2, Po6, Po7, and Po8 which were studied for only 2 yr (2007-2008). We focused on larvae of 6 amphibian species (*T. vittatus*, *S. infraimmaculata*, *H. savignyi*, *B. viridis*, *R. bedriagae*, and *P. syriacus*) in 14 aquatic habitats spread over an area of approximately 1400 km² in northern Israel. The habitats included 3 springs (Sp1, Sp2, and Sp3) and 1 stream (St1) which were stable water bodies where there was water year round, 2 rock pool holes (Pi1 and Pi2) which were filled by rainwater and their hydroperiods were long (about 200 d/yr), and 8 ponds (Po1-Po8) which were flooded during autumn when rainfall began, and gradually dried out between the late winter months and early summer (Fig. 1). Elevations and GPS coordinates of each location (Table 1) were recorded using a handheld Garmin eTrex GPS (Garmin International, Inc., Olathe, KS, USA).

Water quality testing

Samples were taken every 2 wk during the

period when the pools were filling up (starting from the formation of the water body until it was completely dry). On each sampling date, a pool was characterized by the following variables: (i) water quality testing during larval growth, which was described in detail in a previous study (Goldberg et al. 2009). Water quality included DO (%), water temperature (°C), pH, electrical conductivity ($\mu\text{s}/\text{cm}$), ammonium ions (mg/L), nitrate (mg/L), chlorophyll (chl)-a (mg/L), and aquatic invertebrates, i.e., the number of taxa and biomass ($\mu\text{g}/\text{L}$). Water parameters were analyzed by a one-way analysis of variance (ANOVA), with the level of significance between groups set at $p < 0.05$ (Tukey's test). (ii) Surface area was measured with a measuring tape, depending on the water body shape (a square, circle, ellipse, or undefined). This variable was estimated by measuring the maximum length and width or diameter of the pool and then calculating the flooded percentage of this rectangular area (iii) Water body volume: [1] For a constant depth of water body for each area, the depth was measured at 1 point, and the volume was calculated according to the area multiplied by the depth. [2] For a water body the depth of which was not constant, a stick with a ruler was placed in a bucket of concrete at the deepest point of the area before the rainy season, and the volume was calculated according to the volume of a hemisphere.

Larval period and larvae collection

The larval period is the time when larvae hatched from laid eggs. In this study, the larval period was adjusted by ± 14 days according to the frequency at which we arrived at the breeding sites. Once every 2 wk, 5-7 tadpoles of each species from a depth of approximately 10-120 cm were captured with a dip-net. This capture was made at any time at 3 different points in the pool (with totals of about 15 individuals of each species) (Heyer et al. 1994). The full length of each larva was measured with calipers (± 0.5 mm), and each tadpole was immediately released at the point of capture without marking.

Larval growth model and statistical analysis

Differences in larval sizes and time periods that larvae had spent in the water body until they reached metamorphosis were calculated according to the Von Bertalanffy growth function (VBGF) (Von Bertalanffy 1957):

$$L(t) = L_{\infty} - (L_{\infty} - L_0)e^{-kt},$$

where L_0 is the fixed average length at birth ($t = 0$) and was estimated by the mean of the smallest individual caught in all populations for each species; L_{∞} is the mean maximum length ($t = \text{infinity}$); $L(t)$ is body size at age t ; and k is a growth coefficient which defines the shape of growth with units of reciprocal time (e.g., 1/d). Knowledge

of the size at hatching allowed application of this model to amphibians (Hemelaar 1988, Arntzen 2000). The growth rate can be calculated with the following equation:

$$\text{Rate} = \frac{dL}{dt} = (L_{\infty} - L(t))k,$$

which is maximal when $L(t)$ is minimal. The model



Fig. 1. Fourteen breeding sites of amphibian larvae examined in this study: St1, Tel-Dan Spring; Sp1, Balad Spring; Sp2, Humema Spring; Sp3, Navoraya Spring; Po1, Manof Pond; Po2, Kash Pond; Po3, Dovev Pond; Po4, Matityahu Pond; Po5, Lehavot Pond; Po6, Sasa Pond; Po7, Fara Pond; Po8, Raihania Pond; Pi1, Maalot rock pool hole; Pi2, Nimrod rock pool hole.

was calculated for each species at each breeding site, depending on the appearance of different species in the habitats.

For each species, comparison of larval lengths in different ponds at metamorphosis was made. Furthermore, larval ages at metamorphosis (*d*) in the different ponds were compared. These 2 parameters, larval length and larval age, were analyzed by a one-way ANOVA, with the level of significance between groups set at $p < 0.05$. In addition, we compared the average length of larvae of *S. inframaculata* and the average age

at metamorphosis in permanent and ephemeral breeding sites by a *t*-test.

RESULTS

Volumes of water that accumulated in each habitat and their hydroperiods are shown in table 2. Habitat Po6 had the maximum volume of water, while Sp2 had the minimum volume. Humema spring (Sp2), Tel-Dan stream (St1), Maalot rock pool hole (Pi1), and Nimrod rock pool hole (Pi2)

Table 1. Location of sampling

Label	Site	Type	Longitude (E)	Latitude (N)	Residential/ Agricultural areas	Elevation (m)
Sp1	Balad	spring	35°04'17"E	32°43'13"N	nra	446
Sp2	Humema	spring	35°23'43"E	33°00'28"N	-	900
Sp3	Navoraya	spring	35°30'39"E	32°59'47"N	-	663
St1	Tel-Dan	stream	35°39'10"E	33°14'53"N	-	190
Pi1	Maalot	rock pool hole	35°16'26"E	33°00'06"N	nra	596
Pi2	Nimrod	rock pool hole	35°42'48"E	33°15'05"N	-	760
Po1	Manof	pond	35°13'52"E	32°50'58"N	nra	340
Po2	Kash	pond	35°29'26"E	33°01'47"N	naa	809
Po3	Dovev	pond	35°24'54"E	33°03'05"N	naa	765
Po4	Matityahu	pond	35°27'18"E	33°04'04"N	naa	665
Po5	Lehavot	pond	35°38'28"E	33°09'13"N	naa	70
Po6	Sasa	pond	35°23'30"E	33°01'58"N	naa	810
Po7	Fara	pond	35°27'39"E	33°03'58"N	naa	676
Po8	Raihania	pond	35°29'10"E	33°03'01"N	nra/naa	665

nra, near residential areas; naa, near agricultural areas.

Table 2. Mean values and standard deviation (S.D.) of biotic and abiotic water quality parameters measured at each breeding site

Site	Aquatic invertebrates				
	Taxa (no.)	Mean biomass ($\mu\text{g/L}$)	Range of chlorophyll <i>a</i> (mg/L)	Mean hydroperiod (<i>d</i>)	Mean maximum volume (m^3)
Sp1	9	2.8×10^2	0.3- 97.0	ay	19 (4)
Sp2	0	0	0.1- 2.4	ay	0.18 (0.07)
Sp3	11	2.6×10^4	0.2-148	ay	6 (0.3)
St1	8	8.5×10^3	0.1- 28.8	ay	1.3 (0.1)
Pi1	2	75	0.2- 2.9	227 (51)	9 (2)
Pi2	13	9.9×10^3	0.3-219.9	ay	162 (14)
Po1	7	1.4×10^4	1.0- 95.7	139 (26)	49 (11)
Po2	6	1.2×10^5	1.7-222.4	172 (8)	2335 (444)
Po3	8	3.3×10^3	1.9- 89.2	183 (16)	278 (31)
Po4	10	5.9×10^4	0.7- 43.1	210 (4)	4839 (847)
Po5	6	2.3×10^4	1.0- 65.1	130 (19)	10272 (2291)
Po6	7	1.7×10^5	1.3-347.7	215 (18)	11463 (1559)
Po7	8	9.6×10^5	0.9-581.7	297 (54)	1109 (107)
Po8	7	3.2×10^5	0.6-237.0	271 (18)	7956 (390)

ay, all year.

were inhabited only by salamander larvae, which means that *S. infraimmaculata* larvae were observed in all types of breeding sites. All other breeding sites were inhabited by larvae of several amphibian species in various community structures (according to larval species) and at various times throughout the breeding season (Table 3). *Hyla savignyi* and *R. bedriagae* larvae were found in both ephemeral ponds and springs. Occasionally, there were a few overlapping periods in which larvae of *S. infraimmaculata* were observed with

larvae of *H. savignyi*, *R. bedriagae*, *T. vittatus*, and *B. viridis* (Table 3). *Bufo viridis*, *P. syriacus*, and *T. vittatus* were only found in ephemeral ponds and often throughout the growth period usually with an overlap in the presence of these 3 species and *H. savignyi* (Table 3). Water-quality data were measured for each species as shown in figure 2. *Salamandra infraimmaculata* larvae were found under extreme conditions of water temperature (an average of 13°C), chl-a concentration (an average of 10.7 mg/L), and nitrate concentration

Table 3. Overlap times when different larval species were in the same habitat

Site	Species	Months when different larval species were in the same habitat									
		Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	June	July
Sp1	<i>Si</i>	•	•	•	•	•	•	•	•		
	<i>Hs</i>								•	•	•
Sp2	<i>Si</i>	•	•	•	•	•	•	•	•	•	•
Sp3	<i>Si</i>	•	•	•	•	•	•	•			
	<i>Hs</i>									•	
	<i>Rb</i>								•	•	•
St1	<i>Si</i>			•	•	•	•	•	•	•	•
Pi1	<i>Si</i>		•	•	•	•	•	•	•	•	•
Pi2	<i>Si</i>			•	•	•	•	•	•		
Po1	<i>Si</i>		•	•	•	•	•				
Po2	<i>Bv</i>						•	•			
	<i>Hs</i>							•	•		
	<i>Tv</i>						•	•			
	<i>Ps</i>						•	•	•		
Po3	<i>Si</i>				•	•	•				
	<i>Hs</i>							•	•		
	<i>Tv</i>							•	•		
	<i>Rb</i>								•	•	
Po4	<i>Bv</i>						•	•			
	<i>Hs</i>							•	•		
	<i>Tv</i>							•	•		
	<i>Ps</i>						•	•	•		
Po5	<i>Hs</i>						•	•	•		
	<i>Rb</i>								•	•	
Po6	<i>Si</i>				•	•					
	<i>Bv</i>						•	•	•		
	<i>Hs</i>						•	•	•		
	<i>Tv</i>							•	•		
Po7	<i>Ps</i>							•	•		
	<i>Si</i>			•	•	•	•				
	<i>Bv</i>						•	•	•		
	<i>Hs</i>						•	•	•		
	<i>Tv</i>							•	•	•	
	<i>Ps</i>							•	•	•	
Po8	<i>Rb</i>									•	•
	<i>Bv</i>							•	•		
	<i>Hs</i>						•	•	•		
	<i>Tv</i>							•	•	•	
	<i>Ps</i>							•	•	•	•

Si, *Salamandra infraimmaculata*; *Hs*, *Hyla savignyi*; *Rb*, *Rana bedriagae*; *Tv*, *Triturus vittatus vittatus*; *Bv*, *Bufo viridis*; *Ps*, *Pelobates syriacus*.

(an average of 2.9 mg/L) compared to the other species. *Rana bedriagae* larvae had the lowest values of oxygen concentration (47%) and pH (8), and the highest values of conductivity (1052 μ S/cm)

and ammonium ions (1 mg/L) compared to the other species. Both *S. infraimmaculata* and *R. bedriagae* inhabited ephemeral and permanent habitats. The salamander was present from the

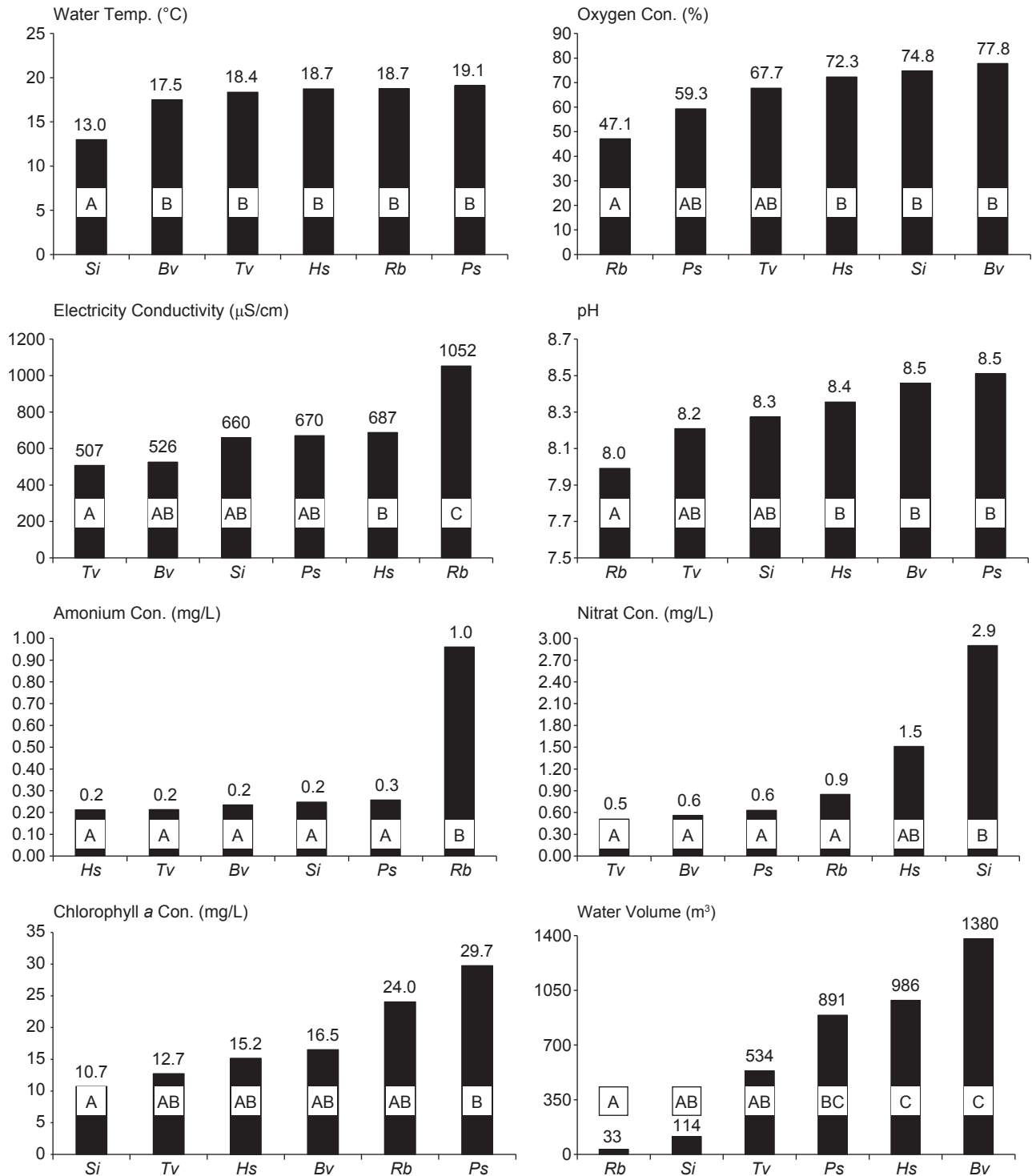


Fig. 2. Mean values of several water parameters measured for each species. Columns that share a letter do not statistically significantly differ from one another (ANOVA followed by Tukey's test; alpha = 0.05).

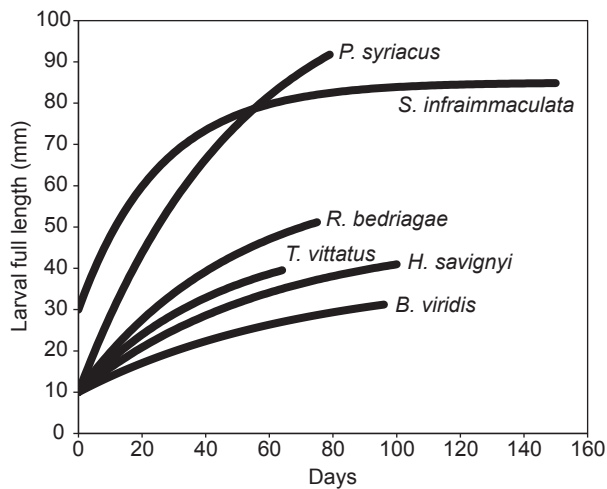


Fig. 3. Growth rates of larvae of *Salamandra infraimmaculata*, *Triturus vittatus*, *Hyla savignyi*, *Rana bedriagae*, *Bufo viridis*, and *Pelobates syriacus* found in 14 ephemeral and permanent habitats during 2005-2008. Curves show the Von Bertalanffy models of growth. The Y axis shows larval size in full length (mm), and the X axis shows time periods in the number of days that larvae had spent in the water body until they reached metamorphosis.

early breeding season, and the frog was present from late spring (Table 3). Four other species inhabited only ephemeral habitats (except *H. savignyi*) on overlapping dates and when water-quality parameters measured in them did not usually significantly differ (Fig. 2).

We modeled twice the rate of larval growth in the habitats studied according to the Von Bertalanffy model of growth: (i) 1 curve for each species represented the average growth rate of species in habitats in the study (Fig. 3) and (ii) another calculation showed the growth curves of tadpoles among individuals of the same species in different habitats (Table 4, Appendix). Within each growth curve, the tangent of the model was higher for larvae that reached larger sizes. These calculated growth model formulas not only varied with the breeding site, but some with the year during which the same species were monitored in a particular habitat. In habitats where water was available year round and the water quality was relatively constant, growth that took place over a longer period was compared to that at ephemeral

Table 4. Summary of mean values and standard deviation (SD) of the age and length at metamorphosis of amphibian populations in 14 breeding habitats

Species	Ephemeral/ Permanent habitats	Mean age at metamorphosis (d)	Mean maximum length at metamorphosis (mm)	Mean growth coefficient (1/d)
<i>Si</i>	Ephemeral habitat	106.58 (20.36)	56.7 (10.4)	0.006 (0.0015)
	Permanent habitat	206.73 (33.43)	48.3 (8.7)	0.006 (0.0011)
	Rock pool hole	207.75 (32.70)	57.0 (13.7)	0.017 (0.0024)
<i>Hs</i>	Ephemeral habitat	76.33 (15.89)	36.8 (7.6)	0.072 (0.0151)
	Permanent habitat	79.53 (17.21)	35.4 (3.0)	0.005 (0.002)
<i>Rb</i>	Ephemeral habitat	61.83 (12.13)	46.1 (12.3)	0.049 (0.0068)
	Permanent habitat	77.50 (10.61)	36.3 (9.2)	0.032 (0.023)
<i>Tv</i>	Ephemeral habitat	60.33 (20.93)	35.2 (6.2)	0.064 (0.036)
<i>Bv</i>	Ephemeral habitat	64.50 (23.53)	33.1 (5.0)	0.021 (0.008)
<i>Ps</i>	Ephemeral habitat	66.7 (15.87)	83.6 (19.5)	0.020 (0.0023)

Si, *Salamandra infraimmaculata*; *Hs*, *Hyla savignyi*; *Rb*, *Rana bedriagae*; *Tv*, *Triturus vittatus vittatus*; *Bv*, *Bufo viridis*; *Ps*, *Pelobates syriacus*.

breeding sites. This phenomenon was particularly evident in tracking tadpoles of *S. infraimmaculata* (Table 4). In addition, it was found that *S. infraimmaculata* larvae that inhabited rock pool holes had a mean age at metamorphosis similar to data obtained from permanent habitats and had a mean length at metamorphosis similar to data obtained from ephemeral habitats (Table 4).

The size range of tadpoles from hatching to complete metamorphosis, the range of the number of days the tadpoles spent in water, and the ratio of the shortest duration to the longest duration the tadpoles spent in water are shown in table 5. During 4 yr of the study, large gaps were measured by comparing the shortest to the longest durations of all species of larvae. Salamander tadpoles showed the highest ratio (1:4), which may imply greater phenotypic plasticity (Table 5).

Significant differences were observed when

comparing larval lengths at metamorphosis of *S. infraimmaculata* at 10 ephemeral and permanent aquatic breeding sites and the number of days salamander tadpoles spent in water in those habitats. Significant differences were observed when comparing larval lengths at metamorphosis of *R. bedriagae*, *B. viridis*, and *T. vittatus* at 4, 5, and 6 breeding sites respectively. No significant differences were observed when comparing the durations of metamorphosis of *T. vittatus*, *R. bedriagae*, *H. savignyi*, *B. viridis*, and *P. syriacus* (ANOVA, Table 6).

The *t*-test for comparing tadpoles of *S. infraimmaculata* (age at metamorphosis and larval length) in permanent habitats with tadpoles in ephemeral habitats showed significant differences in the number of days tadpoles spent in water ($p < 0.05$) but no significant difference in larval lengths at metamorphosis ($p > 0.05$).

Table 5. Range of tadpole sizes from hatching to complete metamorphosis, and the range in the number of days tadpoles spent in water

Species	Range of tadpole lengths (mm)	Range of ages at metamorphosis (d)	Ratio of shortest to longest duration
<i>Salamandra infraimmaculata</i>	25.3- 93.7	69-273	1:4
<i>Triturus vittatus</i>	7.1- 55.2	32-100	1:3
<i>Hyla savignyi</i>	9.4- 58.6	52-101	1:2
<i>Bufo viridis</i>	11.2- 44.5	30-98	1:3
<i>Rana bedriagae</i>	10.3- 70.6	45-85	1:2
<i>Pelobates syriacus</i>	10.1-127.1	43-90	1:2

Table 6. Examination of statistical significance of larval length at metamorphosis and the number of days tadpoles spent in water (ANOVA, $p < 0.01$). For each species, comparisons were made between water bodies where the species was identified

Species	Parameters tested	ANOVA		
		d.f.	F	Significance
<i>Salamandra infraimmaculata</i>	Age at metamorphosis (d)	9, 35	18.353	***
	Length at metamorphosis (mm)	9, 35	4.362	**
<i>Triturus vittatus</i>	Age at metamorphosis (d)	3, 10	2.299	0.164
	Length at metamorphosis (mm)	3, 10	5.382	*
<i>Hyla savignyi</i>	Age at metamorphosis (d)	7, 18	2.149	0.124
	Length at metamorphosis (mm)	7, 18	0.981	0.490
<i>Bufo viridis</i>	Age at metamorphosis (d)	4, 9	1.781	0.270
	Length at metamorphosis (mm)	4, 9	5.027	*
<i>Rana bedriagae</i>	Age at metamorphosis (d)	2, 6	1.245	0.380
	Length at metamorphosis (mm)	2, 6	54.400	***
<i>Pelobates syriacus</i>	Age at metamorphosis (d)	4, 9	0.687	0.631
	Length at metamorphosis (mm)	4, 9	1.501	0.329

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

DISCUSSION

In the current study, we found variations in size and age at metamorphosis of tadpoles among individuals of the same species in different habitats in a relatively small area. We consider that these variations allow species to complete metamorphosis in habitats with extreme conditions, occupy more habitats, and become more widely distributed.

Several studies examined the effects of different ecological conditions of habitats on the growth of larvae of 4 frogs and 2 salamanders found at various breeding sites in Israel, but those previous studies examined 1 species, e.g., *S. infraimmaculata* (Warburg et al. 1979) or 1 habitat with various species (Degani 1982 1986). In the current study, we followed the larval growth of various species found at 14 breeding sites over a period of 4 yr. Another difference between this study and others is that ecological conditions of the breeding sites of the current study were tested (Fig. 2). This situation may help us better understand the suitability of amphibian larvae to breeding sites.

In the current study, growth was estimated as the change in the mean total length of samples of larvae between sampling dates, as was done by Von Bertalanffy (1957). This apparent growth rate might not coincide with the actual individual growth rate for the following reasons: (i) cases in which the apparent growth rate is influenced by mortality that does not act randomly with respect to size. Some causes of death may be related to a proximity to residential or agricultural areas (Table 2). (ii) In pools in which 2 clusters of tadpoles of different ages coexisted, tadpoles in the late-hatching group grew more slowly than small tadpoles in pools lacking large tadpoles (Kam et al. 2001). (3) Omitting samples of the fastest-growing individuals in a population that had already metamorphosed biases the growth curve downward (Bell and Lawton 1975). In addition, many factors, including environmental quality, trophic interactions (Alford 1999), the density of conspecific larvae (Wilbur 1976), and food resources (Warburg 2009) were suggested as being proximal causes of variations in size and age at metamorphosis of tadpoles. When abiotic and biotic conditions provide performance benefits during the larval stage, metamorphic size is often larger, and larval duration is commonly shorter, as was found in the current study and as supported by previous publications based on

natural environments (Degani 1982 1986) and laboratory conditions (Degani and Mendelssohn 1978, Degani 1993). This leads to further fitness benefits, such as reaching reproductive maturity at an earlier age than slowly growing individuals (Smith 1987, Hawley 2010). In addition, larvae in more-permanent environments reach larger sizes than larvae in ponds with brief hydroperiods (McMenamin and Hadly 2010). Those results are not fully compatible with the current research findings, as described below.

Many amphibian species, especially those inhabiting unpredictable environments, exhibit phenotypic flexibility with regard to growth rates prior to metamorphosis. Flexibility in the timing of metamorphosis can be adaptive, as it allows larval amphibians to respond to changes in the quality of their aquatic habitat (Stearns 1989, Newman 1992) and optimize the probability of successful emergence from the larval environment (Semlitsch and Caldwell 1982, Denver 1997). Results of the current study support these findings especially under the extreme conditions prevailing at the southern extreme of the ranges of most of the amphibian species studied here.

Salamandra infraimmaculata is the only species for which significant differences were observed by comparing larval periods and durations of metamorphosis. It is an endangered amphibian, a Urodela species, that inhabits predictable and unpredictable xeric environments at the edge of its zoogeographic distribution in Israel. A Mediterranean climate prevails in the area studied where summers are long, hot, and dry. This area is characterized by early-Oct. rains of short duration which form rock pools in which this salamander breeds. Since these shallow ponds dry out rather quickly and with additional risks of cannibalism (Lehtinen 2004), larvae have limited time to develop and metamorphose (Warburg 2009). It is highly important that they reach this stage of development quickly and attain the largest size possible. Nevertheless, some predictable habitats and rock pool holes contain water for more than 200 d (Table 5), and tadpoles with a slow growth rate were found over a lengthy period.

In the current study, it was shown that in a number of breeding sites, e.g., Sp2 and Pi1 inhabited by tadpoles of *S. infraimmaculata*, the growth period lasted for over 200 d, while the body size at the end of metamorphosis was relatively small (Appendix). These habitats generally had fewer food resources, and were completely

dark and very shallow, and water temperatures remained below 20°C for long periods (Degani et al. 2007, Goldberg et al. 2009). This observed reduction in growth with longer larval periods, and a smaller size at metamorphosis might be explained in 2 ways: (i) although food resources at these breeding sites were limited (Table 2) and cannibalism was common (Segev et al. 2010), the lengthy stay in the aquatic phase allowed continued growth, albeit at a slow rate; and (ii) the presence of water for an extended period of time, even at the expense of a lack of food and exposure to the danger of predation, is preferable to dealing with extreme heat conditions on land. Therefore, less time is left for post-metamorphs to deal with dry summer conditions on terrestrial habitats.

The 5 other amphibian species (*R. bedriagae*, *B. viridis*, *T. vittatus*, *H. savignyi*, and *P. syriacus*) mainly inhabited ephemeral water bodies (Gafny 1986, Degani and Kaplan 1999, Pearlson and Degani 2008), and there was overlap during their stay in the water bodies. These water bodies were very rich in food (Table 2) (Degani and Mendelsohn 1978) and dried up quickly at the end of the rainy season. Delays in metamorphosis can be lethal to tadpoles in rain pools that dry up. In addition, these 5 species populated habitats which were all located in close proximity to residential or agricultural areas (Table 1), which may have caused high prevalences of amphibian deformities due to exposure to industrial and/or pesticide runoff (Ouelle et al. 1997).

Rana bedriagae inhabited breeding sites from May when water temperatures were relatively high. During this period, the water dehydration rate was fast, and adaptive phenotypic plasticity allowed tadpoles to exert fine control over larval length (significant differences) in response to the poor water quality (Fig. 2). No significant differences were observed when comparing the duration of metamorphosis in these habitats (mostly winter ponds). Loman (2003) demonstrated research results with *R. temporaria* tadpoles grown from populations in 8 source ponds (four of which were temporary and the other four of which were permanent) in southern Sweden, in a common garden experiment. No significant effect of source pond hydroperiod was found, although tadpoles from temporary ponds were expected to exhibit faster development rates than those from permanent ones.

Bufo viridis is an opportunistic breeder with a wide margin of variability in annual reproductive cycle patterns; durations of the reproductive

season varied between populations in the same year and between different years for the same population (Sicilia et al. 2007). We found tadpoles of *B. viridis* in 5 ephemeral aquatic breeding sites for durations of 30-98 d (Table 5) (Degani 1982-1986). Although, no significant differences were observed when comparing the age at metamorphosis, there were significant differences in larval lengths at metamorphosis. A short time to metamorphosis (21.1-31.8 d) was recorded as an adaptation to desert environments in temporary ponds in Egypt (Hussein and Darwish 2000).

Water-quality testing indicated that tadpoles of *T. vittatus* could withstand the lowest values of conductivity (507 $\mu\text{s/cm}$) and nitrate (0.5 mg/L) (Fig. 2). High values of these parameters indicate poor water quality and characterized the end of the breeding season, when the volume of winter ponds rapidly decreased. It was established that *T. vittatus* preferred ephemeral breeding sites during early spring (Table 3), and no significant differences were observed when comparing the duration of metamorphosis. In contrast, as found for the salamander, frog, and toad, there were significant differences in larval lengths at metamorphosis.

Pearlson (2011) noted a high growth rate of *T. vittatus* tadpoles in unpredictable habitats where high ammonium concentrations (of about 6 mg/L) were measured. The high concentrations were caused by a high dehydration rate of the pool, so an increased rate of growth could prevent the risk of death that exists with high ammonium concentrations (Ortiz et al. 2004).

Hyla savignyi inhabited nine of the breeding sites, most of which were ephemeral ponds, and there were no significant differences in the timing of metamorphosis or the final size of tadpoles at metamorphosis. Another study done in Israel (Blaustein et al. 1999) reported that solar radiation, which influences temperature and hydroperiod, should strongly influence growth rates of *H. savignyi* tadpoles in artificial temporary pools. In ca. 200-m ponds on a savanna-like open-park, forested, south-facing slope, development of tadpoles was significantly faster than in ponds on a heavily forested, north-facing slope, although the size at metamorphosis was greater in the latter environment.

There were no significant differences in the size of tadpoles of *P. syriacus* or the duration of metamorphosis. Results of the phenotypic plasticity experiment showed that *P. syriacus* tadpoles in the northern frontier of the distribution

area (Dobrudja, Bulgaria) were able to respond to the rate of water level decrease by speeding up metamorphosis and reducing developmental time accompanied by a smaller metamorphic size (Székely et al. 2010).

The ponds studied had several environmental gradients that could influence tadpole growth and development rates including the pond hydroperiod, water quality, etc. In the study area (and in other parts of their distributions), the amphibian species examined frequently breed in ponds that regularly dry up shortly after the rains stop. This may cause catastrophic mortality, but tadpoles counter this by accelerating development when subjected to cues suggesting that a pond is drying out. *Salamandra infraimmaculata* tadpoles showed the greatest phenotypic plasticity as determined by comparisons of the length and age of tadpoles at metamorphosis during the period of growth in their habitats.

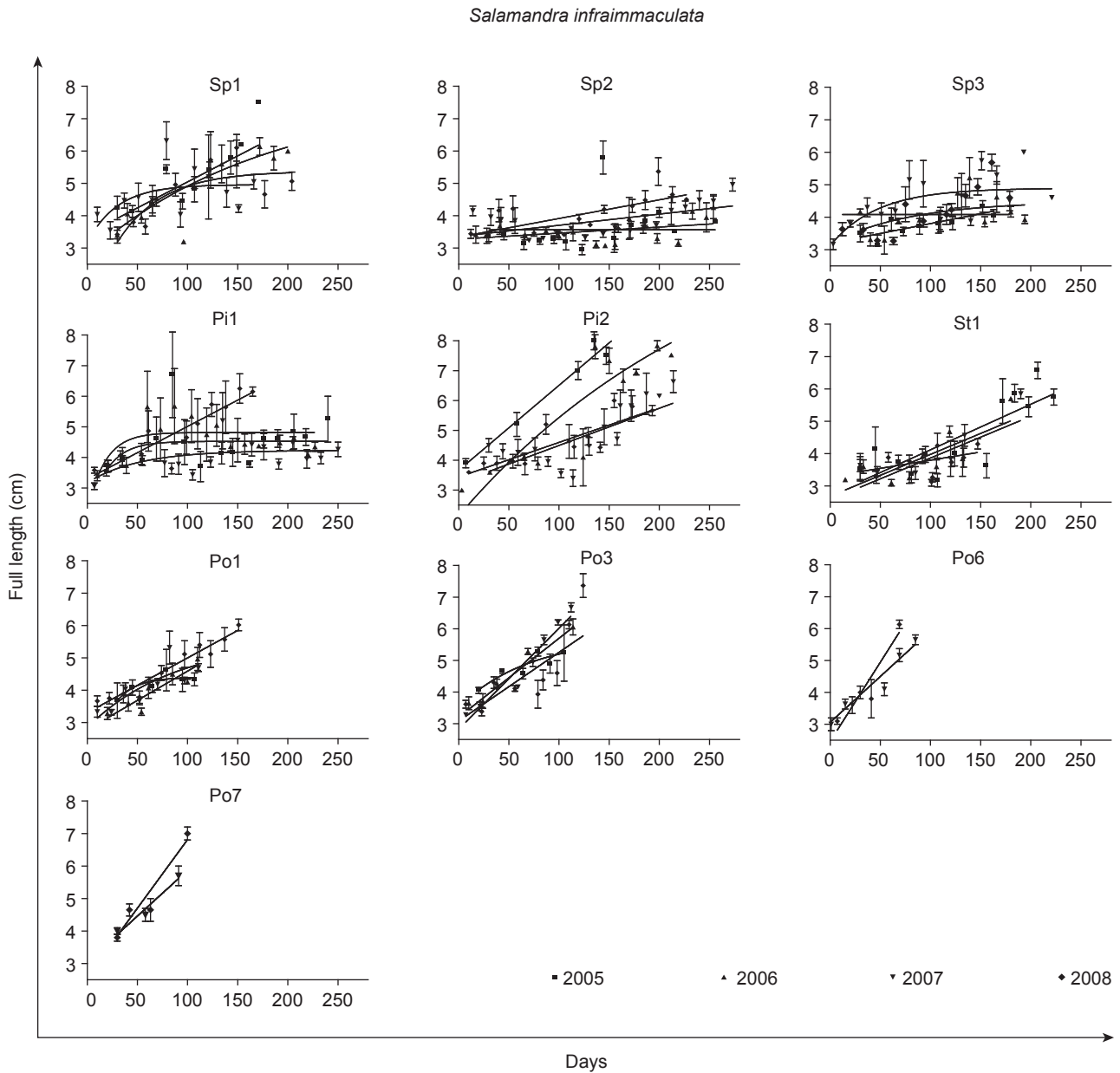
Our findings in natural environments provide important insights into both the degree of plasticity and proximal environmental cues operating in the response of tadpoles of *S. infraimmaculata*, *T. vittatus*, *H. savignyi*, *B. viridis*, *R. bedriagae*, and *P. syriacus* to ephemeral and permanent habitats at the extremes of their distribution ranges.

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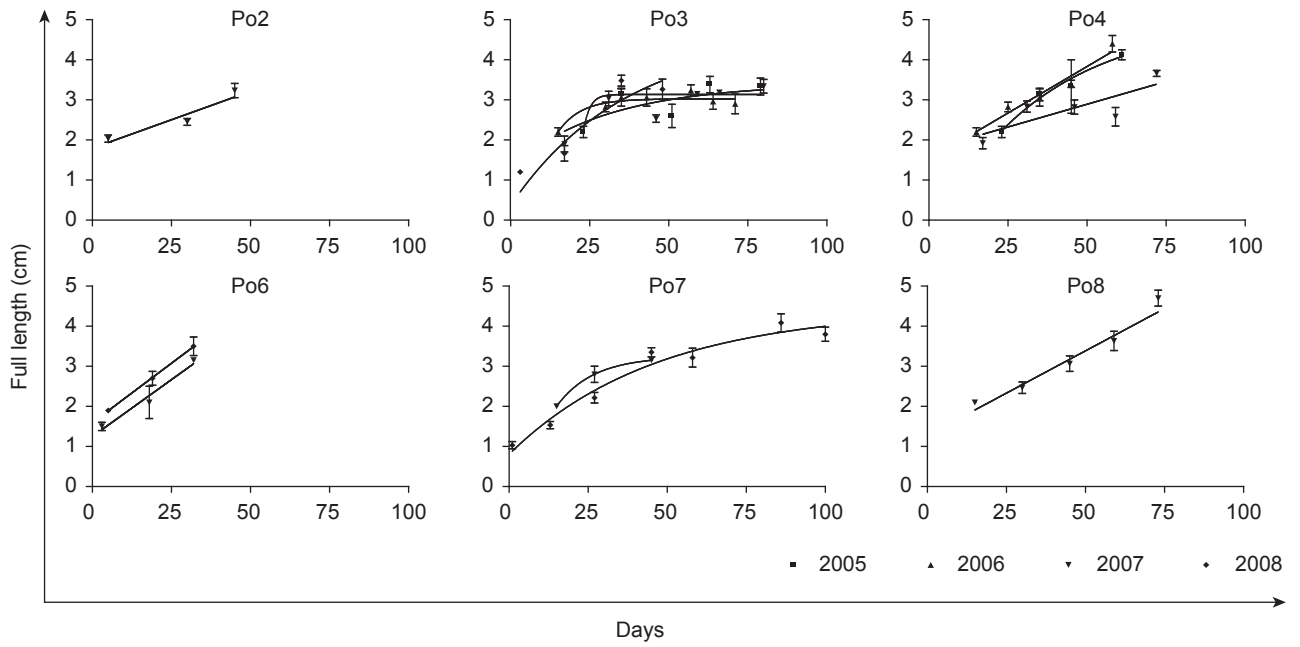
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APPENDIX. Growth of larvae of *Salamandra infraimmaculata* (Si), *Bufo viridis* (Bv), *Hyla savignyi* (Hs), *Rana bedriagae* (Rb), *Pelobates syriacus* (Ps), and *Triturus vittatus vittatus* (Tv). Curves show the Von Bertalanffy models of growth. The Y axis shows larval size in full length (cm), and the X axis shows time periods in the number of days that larvae had spent in the body of water until they reached metamorphosis



Triturus vittatus



Bufo viridis

