Differential growth identified in salamander larvae half-sib cohorts: Survival strategy?

Miriam Cohen, Daniella Yeheskely-Hayon, Michael R. Warburg,* Dana Davidson, Gili Halevi and Rakefet Sharon

Department of Biology, Technion-Israel Institute of Technology, Haifa 32000, Israel

In this study we describe the growth of several different larval cohorts (i.e. half-siblings of the same mother born on the same day) of a rare, xeric-adapted salamander *Salamandra s. infraimmaculata* Martens, 1885, under constant density and food conditions from birth to metamorphosis. The larvae spend the critical first phase of their lives in water, mostly in temporary ponds. Age and weight at metamorphosis were highly affected by varying food conditions. We have identified six different growth modes that these larvae use, both fast growing and slow growing. Each larval cohort was found to use 2–4 different such growth modes regardless of their initial weight. Fast growing modes (I–III) will enable larvae to survive dry years, and metamorphose bigger. Slow growing modes (IV–VI), used by 8% of the larval population, will enable survival only in rainy years. These last growth modes effect differential temporal dispersal in wet years by delaying the emergence of postmetamorphs onto land. Distribution of growth modes in the larval population is affected by food but not by density conditions. Late-born, fast-growing larvae will have an advantage in dry years being able to metamorphose and disperse, whereas the slow-growing larvae will survive only in wet years.

Key words: complex life cycle, niche, pond duration, *Salamandra* larval growth.

Introduction

The rare amphibian salamander, *S. s. infraimmaculata*, inhabits a xeric habitat in a fringe area in northern Israel within the south-easternmost limit of its Palaearctic distribution (Degani & Warburg 1978; Warburg 1997). In this habitat on Mt. Carmel winter rainfall is infrequent, unpredictable and varies greatly in magnitude from year to year, resulting in extremely difficult conditions for an amphibian. Rain pools contain water for only a limited time, thereby limiting the time available for completion of metamorphosis, dependent on dehydration of the pond (Warburg 1992, 1997). Consequently, the timing of onset of breeding is crucial because larvae need at least 5 weeks to complete metamorphosis. Ponds in this habitat fill for the first time early in the rainy season (October–November) and tend to dehydrate for a period of up to 3 weeks before rains continue (Warburg 1986a, b). This causes an additional constraint on aquatic life in the pond,

especially on the newborn larvae (Warburg 1992, 1994). The ponds are at the beginning of the season very poor in invertebrate life and therefore food is scarce (Warburg *et al.* 1978/1979). Later on, additional salamander larvae cohorts add to the food chain and enrich food sources, thereby enhancing cannibalistic predation by other larvae (Warburg 1992; Cohen *et al.* 2005).

It is well known that the premetamorphosis (first 3–4 weeks) of amphibian larvae is entirely dedicated to growth (Etkin 1963), with differentiation followed by the pro-metamorphosis period (at 5–6 weeks of age). During that period, the three main organs involved in physiological adaptation of amphibians to terrestrial life (skin, lungs and kidneys) differentiate and become functional as terrestrial breathing and excretion organs at metamorphic climax (Warburg 1997; Gealekman & Warburg 2000).

Under these circumstances, it is of utmost importance for a larva to grow at a rapid rate during the premetamorphic period, thus gaining advantage in case of pool dehydration (Semlitsch 1987a). Fast growth is essential to achieve metamorphosis as soon as possible and at the largest size.

Complex life cycles and niche shift

Life-history theory is concerned with investigating and explaining by examination of the variations in

life-history traits (Crump 1989a). Reproductive strategies can vary in response to environmental conditions. Different life-histories are an ideal way to spread the risk of reproductive failure which may be a result of unpredictable duration of an unfavorable environment (Lampert & Linsenmair 2002).

Many aquatic species, including amphibians, have a complex life cycle (CLC), a term coined by Wilbur (1980) for such animals that undergo a dramatic change during their lifecycle (most insects not only aquatic). These changes include ontogenetic changes in the individual's morphology, physiology and behavior associated with a change in habitat. CLCs are abundant in the animal kingdom or ubiquitous and they have adaptive mechanisms allowing each phase to respond independently to different selective forces (Moran 1994). It can be viewed as an adaptation that allows a species to exploit two or more different ecological environments (Wilbur & Collins 1973; Wassersug 1975).

The main ecological feature of CLC is the shift in niches that occurs at metamorphosis (Werner & Gilliam 1984; Werner 1986). Larvae and adults of organisms undergoing CLC evolve somewhat independently of each other having different body plans (Werner 1986; Ebenman 1992). Animals may change their use of resources and their niches during their lifecycle, utilizing different niches during different life stages (Ebenman 1992). Thus, alternate phases occupy different niches where each alternating phase has a separate niche (Moran 1994). Consequently, an ontogenetic niche shift takes place (Moran 1994). Such species occupy two different niches; one by the adult (a terrestrial niche) and the other by the larva (an aquatic niche). They are different animals in many respects but belong to the same species having the same genome, each stage occupying one niche.

Unpredictable pond duration and reproductive strategies

MacArthur (1972) discussed the adaptations to unpredictable climate, especially rainfall irregularity. The main problem facing an animal is to 'decide' what to do (his example: hibernation, not knowing if the climate will require doing so).

Amphibians breed in response to rainfall that forms ephemeral pools (Marsh 2000). These are bodies of water that form in a matter of hours (Crump 1989a, b). They are characterized by a hydro-cycle of drying and filling (Lips 2001) that are unpredictable in duration (Crump 1989b), but have a predictable annual dry phase lasting 3–8 months between late spring and autumn. The pond's hydro-period is the total number of days a pond is filled annually (Paton & Crouch 2002). Nevertheless, the pattern and amount of rainfall during the rainy season is unpredictable and varies between years (Lampert & Linsenmair 2002).

The unpredictable environment, especially as reflected in the temporal and spatial distribution and cyclic pattern of nutrient availability, are common features of these pools (Alford 1997) and pose a major challenge to amphibians (Griffiths 1997). Thus amphibian larval developmental rates vary in response to pond drying (Crump 1989a). These temporary ponds, despite their small size and being shallow, can provide habitats for important assemblages of rare and endangered freshwater species (Collinson *et al.* 1995), as is the case for *S. s. infraimmaculata*.

The unpredictable wet periods in temporary ponds seem to favor plasticity in reproduction and development of individuals and populations (van Buskirk 2002). The different life-histories are an ideal way to spread the risk of reproductive failure which may be a result of unpredictable duration of an unfavorable environment (Lampert & Linsenmair 2002). It depends on the initial water depth and the frequency and amount of rainfall (Newman 1989). This hydro-period influences both growth, development and metamorphic size (Wilbur & Collins 1973; Wilbur 1988; Crump 1989a; Lips 2001). Moreover, these stagnant water habitats, temporary ponds and pools, have wide diurnal and average range of temperatures (Popham 1965). Increasing temperatures during the dry period although accelerating aquatic larvae development, risk dying as a result of pond increasing desiccation (Warburg 1992; Griffiths 1997). How can a female assess the water holding capacity (WHC) of a pool and its desiccation risk? (Spieler & Linsenmair 1997). The CLC is an adaptation to such unpredictable environments (see Kaitala 1987) that enables exploitation of uncertainties associated with temporary ponds (Griffiths 1997). Such adaptations to climate have moulded life-history (Maiorana 1976).

Developmental plasticity under unpredictable conditions

An individual animal belonging to one stage needs to 'forsee' the needs of its other stage occupying a different niche. Thus, during the larval period, while in its aquatic niche, it needs to be able to reach a state that will enable it to survive as a terrestrial adult. The adult in its terrestrial niche needs to breed during a suitable period and locate a suitable pond in time to enable successful metamorphosis by the aquatic larva. How can this be achieved?

The aquatic larva has two objectives: to grow fast and to metamorphose as soon as possible and at the largest size.

The present paper deals with growth. There is a phenotypic plasticity in growth and development parameters (Crump 1981, 1989a). Travis (1981) noticed extensive phenotypic variations in growth rates even among sibships (Travis 1983a, b). Tadpoles grow at different growth rates. The significance of different growth curves is that during wet winters, even early born, slow-growing larvae will have a chance of survival. During dry years, among late born, only the fast-growing larvae will survive, as only they achieved optimal size at an earlier age (Travis 1981; Harris 1999). Growth rate is an important measure of larval fitness under almost any imaginable environmental regime (Harris 1999), and will have an effect on post-metamorphic performance (Alvarez & Nicieza 2002a).

Amphibians that have a CLC (inhabiting two different media) exhibit plasticity in timing of and in achieving an optimal size at metamorphosis (Hensley 1993). The extent of larval period (i.e. their age) and size at metamorphosis are related to larval growth history (Wilbur & Collins 1973; Beck 1997; Beachy *et al.* 1999; Alvarez & Nicieza 2002a). Density affects time to metamorphosis and food affects both larval growth and size at metamorphosis (Alvarez & Nicieza 2002b). There is a positive correlation between time of pond drying and number of metamorphosing larvae (Semlitsch & Wilbur 1988). Moreover, in desiccating ponds, tadpoles suffered from crowding effects and metamorphosed at a smaller size (Brady & Griffiths 2000). There is a trade-off between minimizing time spent in the pond (reducing risks of predation and dehydration) and maximizing size at metamorphosis, both important for terrestrial survival and future reproduction and thereby maintaining genetic variation for growth rate (Harris 1999).

After metamorphosis, the postmetamorph needs to disperse as soon and as far as possible during the suitable season because it is limited spatially and temporally. This postmetamorphs' dispersal or 'emigration' (Paton & Crouch 2002) depends on both time and size of the juveniles that will enable them to disperse as soon as possible and to maximal distance. The chance of the species' survival depends on its potential as a colonizer.

Our main aim in this study was to clarify the relationships between the aquatic and the terrestrial stages. Can the aquatic stage in fact influence in any way the future of the young postmetamorph terrestrial stage?

In this work, we used only larval cohorts (i.e. litters of half siblings born to one mother and on the same day) in order to minimize the variability. In addition, we decided to analyze growth modes under constant food and density conditions, eliminating inhibitory growth factors as much as possible (Petranka 1989; Walls & Jaeger 1989; Beebee & Wong 1992). Because only a few female salamanders breed in these rock pools each year with only some of them producing sufficiently large cohorts, this experimental study was spread out over a period of 4 years.

Materials and methods

Experimental layout

This study involved 1499 larvae of 17 cohorts (Table 2). Most of them (1479) were used for data of first week weight, some of those for growth study (428) and some for metamorphosis (325). The main study was carried out during the breeding season in the years 1996–1999 and involved 589 larvae belonging to seven cohorts: 20 larvae from cohort 'A'; 62 from cohort 'B'; 130 from cohort 'C'; 126 from cohort 'D'; 52 from cohort 'E'; 130 from cohort 'F'; 37 from cohort

Table 1. Larval weight at first week of life in 16 cohorts

Cohort	Females weight (g)	Number of larvae	Weight at birth (mg)
A	*	*	*
B	118	62	371.6 ± 26.4
C	108.7	130	220.4 ± 17.0
D	93.9	126	228.1 ± 15.1
E	134	52	326.6 ± 53.0
F	126.7	130	310.3 ± 15.8
G	106	36	334.7 ± 24.9
H	*	32	256.3 ± 22.2
I	127.2	113	307.0 ± 37.4
J	84.5	*	324.0 ± 38.7
K	*	74	320.0 ± 40.0
L	*	121	310.0 ± 40.0
M	*	130	270.0 ± 30.0
N	*	30	310.0 ± 40.0
O	*	129	280.0 ± 20.0
P	*	156	300.0 ± 20.0
Q	*	158	250.0 ± 20.0
Total		1479	

Cohorts B, E, F, G, I, J, O, Q were born early, in autumn. Cohorts C, D, H, K, L, M, N, P were born late, in winter. Differences in larval weight at birth do not correlate with either females' weight or number of larvae in the cohort. Regression test between females' weight and average larval weight in the cohort shows no correlation between the two ($R^2 = 0.095$, $P = 0.45$). For testing the relationship between number of larvae in a cohort and larval weight, we used regression analysis only on the nine cohorts that contained more than 100 larvae (which is the average number of larvae for *S. s. infraimmaculata*, Warburg *et al.* 1978/79). No correlation was found between them ($R^2 = 0.03$, $P = 0.657$). *Data not available.
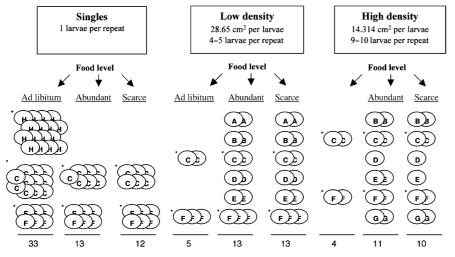
Fig. 1. Experimental layout. Each oval represents one 13.5 cm diameter glass bowl, filled with stale (i.e. aged) tap water 2 cm in depth. Constant density conditions were kept during the experiment by transferring larvae into suitable sized glass bowls. Larvae were fed every 3–4 days with measured amounts of fine chopped beef liver or live *Tubifex* worms (*). Bowls were cleaned 2 h after feeding and filled with fresh water. No difference was found between the effect of food items on either growth or metamorphosis. Scarce food −0.005 g (wet weight) of chopped liver per larva or 0.004 g live *Tubifex* per larva; abundant food −0.02 g (wet weight) of chopped liver per larva or 0.01 g live *Tubifex* per larva; ad libitum – live *Tubifex* worms were present in the bowls during the whole experiment. At the age of 23 days the amount of liver was doubled and at the age of 18 days the amount of *Tubifex* was quintupled. The letter within the oval indicates larval cohorts (A–H). Numbers indicate total number of replicates per treatment.

'G' and 32 from cohort 'H'. Data for weight and length during the first week after birth were available for an additional 910 larvae: cohorts 'I–J' (from I. Warburg pers. comm.), cohorts 'K-O' (Degani & Warburg 1995) and cohorts 'P–Q' (G. Degani, unpubl. data, 1980). Data for age, weight and length at metamorphosis of cohorts 'I–J' was also available. Cohort 'A' was not weighed at birth.

In this study we compared larvae belonging to different cohorts, however, we were limited by the number of breeding females available to us. By using larval cohorts of the same age originating from one mother we had the advantage of limiting size variability among larvae within the cohort (Fig. 3). The variability ranged between 5.1% and 16.2%, averaging 9.6% of the mean weight of larvae at birth (Cohen *et al.* 2005). Experiment layout is given in Figure 1. No pseudo-replicates were used, as each larva was involved only in a single experimental set-up.

Growth

A large number (1479) of larvae were weighed (± 0.01 mg accuracy) and their total length was measured (to the nearest 1 mm) within the first week of their life, out of which 428 larvae were kept under different density and food conditions (see Fig. 1). They were weighed and measured fortnightly and their increase in weight was calculated thereafter for a variable length of time until metamorphosis. The growth increments, for example net gain in weight, were calculated for two periods: the first 3 weeks (W_1) and the following 2 weeks (W_2). Larval length
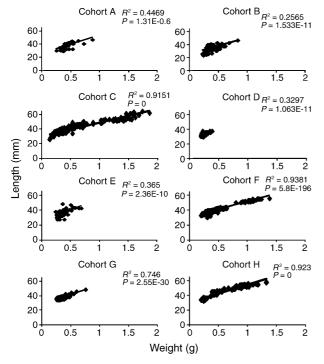
and weight were found to be significantly positively correlated in all cohorts (Fig. 2).

Metamorphosis

We considered metamorphosing larvae with resorption of their gills and their tail fin, and when their larval color changed. At that time weight and length of 325 larvae from seven different cohorts were measured and their ages noted.

Statistical analyses

To reduce variability, all data from each cohort was analyzed separately. Regression analyses were performed on the data of length and weight of eight cohorts in order to find a correlation between these parameters. We also used regression analyses to test the correlation between larval weight at the first week to both mothers' weight and number of larvae in the cohort. For total number of larvae in the cohorts, we used only the larger cohorts in order to minimize variability among the replicates. Two-tailed *t*-test and ANOVA with Student Newman Keuls (SNK) advanced test compared larval weight in the first week between the different cohorts. The *t*-test was used to compare larval size (weight) at the third and fifth weeks between larvae growing in different growth modes. Percentage of ΔW_1 and ΔW_2 were arc-sine square root transformed to meet the parametric assumption of normality and expressed as radians. The transformed values were analyzed by the *t*-test comparing ΔW_1 and ΔW_2 within each growth mode.

Fig. 2. Correlation between weight and length. Larvae size could be quantified by either weight or length. Regression tests revealed a perfect correlation ($P < 0.0001$) between weight and total length dimensions in a total of 477 larvae from eight different cohorts tested (A–H). This enabled us to use only one of those parameters (weight) to represent larval size.

Age and weight at metamorphosis under different food and density treatments were tested using both the two-tailed t-test and ANOVA test with SNK.

Results

Size at first week

Larval size (weight) in 1479 larvae at the first week of their life is rather homogenous within each cohort, but varies significantly ($P < 0.001$) among the different cohorts. The weight varied from 220 mg to 370 mg (Table 1; Fig. 3). Two-thirds of larvae weighed between 250 mg and 350 mg; these differences did not correlate with either the mother's weight or the total number of larvae in a cohort.
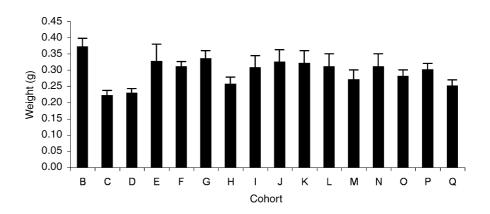
Growth modes

Growth of 428 larvae kept in 114 bowls under different experimental treatments (Fig. 1) was followed for 5 weeks until the first larva had metamorphosed. Based on this data, the percentage of gain in weight was calculated for these time periods (ΔW_1, ΔW_2) in all experimental set-ups. Six different growth modes (I–VI) were identified (Fig. 4a) and can be divided into two main trends:

1. There was a positive increase in weight during both growth periods (I–III). Mode I is characterized by faster growth during the second period. In mode II most weight is gained during the first period, whereas in mode III the growth rate is constant.
2. A loss in weight was apparent at least during one of the two periods (IV–VI). In mode IV, larvae lost weight during the first period, whereas in mode V they lost weight during the second period and in mode VI there was continuous loss of weight. Larval size at the third and fifth week was compared among the different growth modes. When 3 weeks-old, larval size differed significantly ($P < 0.005$) when growing in modes I–III. Larvae growing in mode II were much larger than larvae growing in the other modes. No such difference was found in the fifth week.

Distribution and frequency of growth modes

The most frequent growth modes in the larval population are modes I and II (51%), whereas modes IV–VI are rare (8%) and appear only under extreme conditions (scarce food, Fig. 4b). Regarding the

Fig. 3. Average weight at birth during the first week of life. During the first week of life larval weight is homogenous within cohorts, but differs among cohorts. Significant differences were found between cohorts 'B–H' except between E and G. Detailed data for cohorts I–Q were not available except for mean ± SD. (I, J) Data from I. Warburg (pers. comm.); (K–O) data from Degani and Warburg (1995); (P–Q) data from Degani (unpubl. data, 1980).

Table 2. Distribution and frequency of growth modes

Growth modes	B	C	D	Cohort E	F	G	H
I	(2) D_l/F_a (1) D_l/F_s	(2) D_h/F_a (2) D_h/F_s (2) D_l/F_a (2) D_l/F_s (2) S/F_l (3) S/F_a (1) S/F_s	(1) D_l/F_a (2) D_l/F_s		(1) D_h/F_l (2) D_h/F_a (2) D_h/F_s D_l/F_l (1) (3) D_l/F_a (3) D_l/F_s (5) S/F_l (6) S/F_a (3) S/F_s	D_h/F_a (1) D_h/F_s (2)	(6) S/F_l
II		(2) D_h/F_l (2) D_l/F_l (9) S/F_l (4) S/F_a (3) S/F_s	D_h/F_a (1) (1) D_l/F_a	(2) D_h/F_a D_h/F_s (1) (1) D_l/F_a	D_l/F_l (3) (3) S/F_s		(6) S/F_l
III		(1) S/F_l		(1) D_l/F_a	(1) D_h/F_l	(1) D_h/F_a	(4) S/F_l
IV	(2) D_h/F_s (1) D_l/F_a (2) D_l/F_s				(1) S/F_s		
V			(1) D_h/F_s			(1) D_h/F_s	
VI				(1) D_l/F_s			

Growth modes (I–VI) as in Figure 4. Numbers in parentheses indicate the number of replicates. D_h, density high; D_l, density low; S, singles; F_a, food abundant; F_s, food scarce; F_l, food lib.

Table 3. Statistical analysis of weight and age at metamorphosis

		Scarce C	Scarce F	Scarce E	Scarce A	Abundant D	Abundant C	Abundant F	Ad libitum H	Ad libitum C
Ad libitum	W	*	*	*	*	NS	NS	NS	NS	*
	F Age	*	*	*	*	*	*	*	*	NS
Ad libitum	W	*	*	*	*	*	*	*	NS	
	C Age	*	*	*	*	*	*	*	*	
Ad libitum	W	*	*	*	*	NS	**	NS		
	H Age	*	*	*	*	*	*	*		
Abundant	W	*	*	**	***	NS	NS			
	F Age	*	*	NS	*	*	NS			
Abundant	W	*	*	*	*	NS				
	C Age	*	*	NS	*	*				
Abundant	W	*	*	*	*					
	D Age	**	NS	*	NS					
Scarce	W	NS	NS	NS						
	A Age	**	n.s	*						
Scarce	W	NS	NS							
	E Age	***	NS							
Scarce	W	NS								
	F Age	NS								

Age and weight (W) at metamorphosis in different treatment groups are compared using the *t*-test and SNK test. Both tests indicated that larvae fed ad libitum differed significantly from all those fed scarcely (scarce), but not from most larvae that were given abundant food (abundant). Larvae fed scarcely generally differed significantly from those that were given abundant food. Upper values are *P*-values ($*P < 0.01$; $**P < 0.001$; $***P < 0.0001$) for weight comparison, lower values are for age comparison. NS, not significant.

frequency of the other two growth modes, II and III increases profoundly under improved food conditions (abundant and ad libitum, Fig. 4b). The significance of these findings will be discussed later.

Larvae belonging to the same cohort did not use only one growth mode but rather used 2–4 different growth modes (see Tables 3–5). Moreover, larvae grew using a certain growth mode irrespective of density

Fig. 4. Growth modes. (a) Large plots show actual growth pattern. In order to study growth modes, percentages of weight added during the first 3 weeks (δW_1) and during the following 2 weeks (δW_2) were calculated. Six growth modes were identified: (I) $\delta W_1 < \delta W_2$; (II) $\delta W_1 > \delta W_2$; (III) $\delta W_1 = \delta W_2$; (IV) $\delta W_1 < 0$; (V) $\delta W_2 < 0$; (VI) $\delta W_2 < \delta W_1 < 0$. Significant differences between W_1 and W_2 were found in 'I', 'II', 'IV' modes (*t*-test, details in Table 2). In the small inserts percentage of δW_1 (black) and δW_2 (blank) are presented for the six different growth modes. (b) Pie charts illustrate the frequency distribution of growth modes among larvae tested. Mode 'I' (blank) is the most frequent mode whereas modes 'II' and 'III' increase profoundly under improved food conditions (scarce < abundant < ad libitum). Modes 'IV–VI' appear only under extreme conditions of scarce food and high density (see also Table 2).
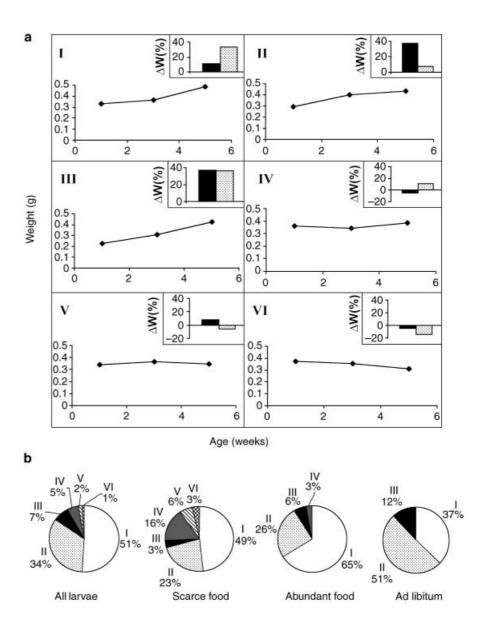
Table 4. Larval growth modes used by different cohorts

Mode	Cohort	Early (autumn)	Late (winter)
I	B,C,D,F,G,H	3	3
II	C,D,E,F,G,H	2	3
III	C,E,F,G,H	3	2
IV	B	1	0
V	D,G		
VI	E	1	0

Early born cohorts: B, E, F, G. Late born cohorts: C, D, H. Conclusions: 1. Both early and late born cohorts use different growth modes. 2. Most cohorts use fast growing modes. 3. Slow growth modes are mostly used by early (autumn) born cohort.

conditions (see C, F with high, low and singles densities grew using the same mode).

Metamorphosis

Both age and weight of metamorphosing larvae were largely affected by food levels (Fig. 5) irrespective of their maternal origin. Larvae fed ad libitum metamorphosed significantly sooner and at a larger size than larvae fed differently, whereas the larvae that were scarcely fed were significantly smaller and metamorphosed later than others (Figs 5 and 6; Table 4). No such effect was seen under different density conditions. There appears to be an optimal weight for metamorphosis (~1.5 g) but if this weight is not reached soon enough, age is the limiting factor.

Table 5. Metamorphosis of early and late cohorts during dry and wet periods

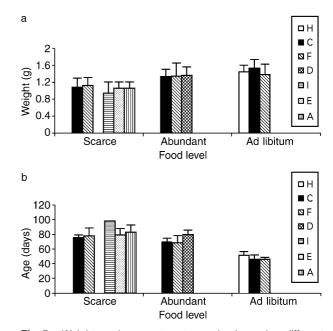| Birth	Early				Late			
Rainfall	Dry		Wet		Dry		Wet	
Growth mode	S	F	S	F	S	F	S	F
Survival	Die	Die	*	**	***	****	***	****

F, fast-growing modes (I–III); S, slow-growing modes (IV–VI). Early, autumn; late, winter. *Born mid-October, slow-growing, metamorphose after 80 days in mid-December; **born mid-October, fast-growing, metamorphose after 45 days at end of November to beginning of December; ***born beginning of December, slow-growing, metamorphose after 80 days beginning of February; ****born beginning of December, fast-growing, metamorphose after 45 days mid-January. Not shown, larvae born very late end of December to beginning of January. Some of them if fast-growing may metamorphose during March provided the pond still contains water, does not bloom and it is not too hot. Conclusion: metamorphosis is spread over a period of 3–4 months (December–March). In wet years, fast-growing larvae even of late-born cohorts will be able to metamorphose provided all early cohorts' cannibalistic larvae have metamorphosed. Slow-growing larvae have the benefit of less competition, abundance of food, and can grow to a large size metamorphosing as large postmetamorphs, provided it is a wet year. Early born larvae have the advantage in metamorphosing early and having up to 4–5 months of feeding and growing eventually dispersing as juveniles. Late-born cohorts have only about 3 months before the onset of summer.
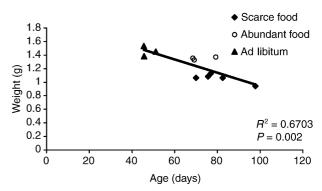
Discussion

S. s. infraimmaculata is a salamander surviving in xeric habitats in a fringe area in Israel at the outskirts of this species south-eastern Palaearctic distribution. It lives under extreme conditions where rock pools form early in the season (October–November) and dehydrate for a period of up to 3 weeks before the next rainy spells that fill the ponds again (in 65% of 25 years of observation; Warburg in prep.).

Short pond duration can cause early metamorphosis (Wilbur & Alford 1985; Semlitsch & Wilbur 1988; Newman 1989; Semlitsch 1990; Resetarits 1996; Denver *et al.* 1998) but only larvae that reached the advanced developmental stage could metamorphose (Smith-Gill & Berven 1979). Thus, the unpredictability of pond duration is the major force acting on the larvae, whereas survival during dispersal is the main challenge to the young postmetamorphs.

Many amphibians, *S. s. infraimmaculata* included, have CLCs implying a shift in their niches during their lifecycle. Selective forces acting on the aquatic larvae occupying an aquatic niche differ from those acting on the postmetamorphs occupying a terrestrial niche. Unless the aquatic larval phase utilizes its plasticity during its growth and the terrestrial adult phase

Fig. 5. Weight and age at metamorphosis under different feeding conditions. (a) Groups of larvae fed scarcely metamorphosed at significantly smaller dimensions than other larvae. Larvae fed ad libitum reached significantly higher weight at metamorphosis. (b) Larvae fed ad libitum metamorphosed significantly sooner than larvae from other groups of treatments but no such clear differences were found between larvae groups raised under abundant and scarce food conditions. Within the groups of treatment, larvae metamorphosed almost simultaneously regardless of their different cohort origin. For statistical analysis of these data see Table 3.

Fig. 6. Weight and age at metamorphosis. There is a reverse linear correlation between age and weight at metamorphosis. Larvae fed ad libitum reached higher weight and metamorphosed sooner than larvae from scarce food groups that metamorphosed smaller and much later.

utilizes all the opportunities available through reproductive plasticity, survival in such an unpredictable, hostile environment will not be possible for the individual salamander hence for the species.

Twenty-five years of observation on a single breeding population have shown that female *S. s. infraimmaculata* have two reproductive strategies: some (50.9% of the population) breed early in the season whereas others (49.1%) breed late (Warburg 1992, 1997 and in prep.). In dry years, early-born larvae will die due to pond dehydration whereas in wet years they will have the advantage of feeding on their sibships (cannibalism in these larvae is discussed elsewhere; Cohen *et al.* 2005).

By studying growth of larval cohorts known to be of the same age and of a single maternal origin (thereby lowering variability), and by calculating the percentage of weight added, we were able to identify different growth modes, which could not have otherwise been identified. In our experimental set-up, food and density conditions remained constant throughout larval life and growth inhibitory factors were eliminated (by changing the water daily). We have seen here that half-sib larvae belonging to a single cohort (born at the same time to one mother) grow by using more than a single growth rate. It was previously shown that even full-sibs show variation in growth rates (Travis 1983a; b). Six different growth modes, half of them fast-growing larvae and half slow-growing larvae, were identified. What is the significance of these findings? Only rapidly growing larvae I–III (92% of the larval population) will have a chance to survive a short period of drought (up to 1 week on wet mud; Warburg 1986b, 1992). Desiccation due to pond drying can be a major component of reproductive failure in some years (Tevis 1966; Shoop 1974; Semlitsch 1987b), therefore growth mode II is crucial for survival.

When the ponds fill up again, larvae using growth mode II will have a great advantage over other larvae in being bigger, thereby having superior ability in obtaining the limited food resources available then (Wilson 1975), and in being capable of preying on others (Warburg *et al.* 1979; Alford 1997; Cohen *et al.* 2005). This results in a very rapid growth during the critical first period of larval life (premetamorphosis) in the pond (Etkin 1963). As a result, these larvae may have an advantage during pro-metamorphosis reaching a more advanced stage during organogenesis of crucial organs (e.g. skin, lungs and kidney), at metamorphic climax (Warburg *et al.* 1994; Warburg 1997; Gealekman & Warburg 2000). Later on this will enable them to feed more frequently and grow even faster (like those fed here ad libitum), reaching metamorphosis bigger and sooner than the other sibships (Smith-Gill & Berven 1979; Crump 1990; Newman 1998). Consequently, they will mature and breed sooner than their siblings that metamorphosed later (April–May). It became apparent that mode II is

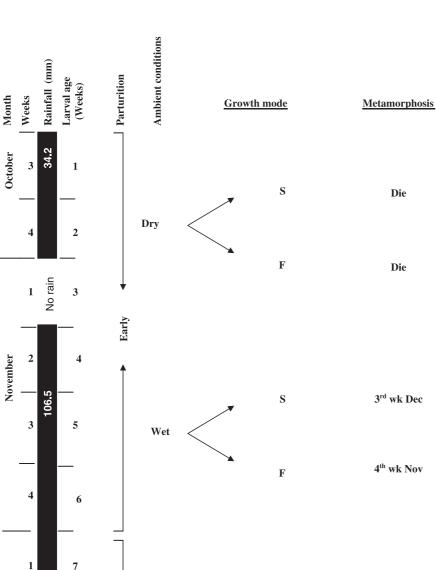of utmost importance for the survival of this species under such extreme conditions.

In rainy years (in 35% of 25 years of measurements) all larvae, including the slow growing growth modes IV–VI, will survive and will most likely metamorphose. Unlike in several other studies (Semlitsch & Caldwell 1982; Newman 1994; Scott 1994), we did not find evidence for density effect on size and age at metamorphosis, possibly because in our study growth inhibiting factors were eliminated from experimental conditions by changing water frequently.

In a model (Fig. 7) we attempt to link data presented in this paper with data accumulated over the years (Warburg 1992, 1994). This model is consistent with both the Travis model (Travis 1984), which predicts that developmental rate is set early in the larval period, and the Wilbur-Collins model (Wilbur & Collins 1973), which predicts adjustments in developmental (growth) rates to changes in environmental (food) conditions. Lastly, our findings that larvae fed ad libitum metamorphosed sooner and bigger than others is consistent with the Smith-Gill model (Smith-Gill & Berven 1979), which asserts that individuals growing most rapidly will probably complete metamorphosis early, regardless of size.

The question remains as to what is the evolutionary reasoning for the persisting presence in the population of slow-growing modes (8% of the larval population). Salamander larvae are born equal in size, grow at different rates and metamorphose at different sizes (Warburg *et al.* 1979). It seems to us that there is an evolutionary advantage in distributing the emergence on land over a period of time, thereby reducing the risk of possible postmetamorphic cannibalism, and timing the dispersal of juveniles at intervals to enable the best chance of survival for the terrestrial postmetamorphs. The six larval growth modes described here are geared to timing dispersal of the postmetamorphs at suitable intervals to enable their optimal chance of survival. Dispersal of young postmetamorphs (also called emigration by Paton & Crouch 2002) is different from the adults' breeding migration (which they call immigration). It is the movement of young animals from their place of birth in search of food or shelter (Lack 1954). The first larvae to metamorphose in January (mid-winter) have a better chance of dispersing and locating a site suitable for sheltering during the hot, dry summer.

Fig. 7. Model describing salamander larval growth during dry and wet autumns and winters. On the left, time scale that indicates weeks of the months, average rainfall (in mm) and larval age (in weeks) relative to the growth presented in the flow chart. F, fast growing modes (I–III) metamorphosing within 45 days; S, slow growing modes (IV–VI) metamorphose within 80 days; early, autumn; late, winter; not shown, larvae born very late (end of December to beginning of January). Some of them, if fast-growing, may metamorphpose during March provided the pond still contains water, does not bloom, and it is not too hot. Metamorphosis is spread over a period of 4–5 months (November–March).

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