

Age and size at metamorphosis of *Salamandra infraimmaculata* larvae born in the laboratory and raised under different density regimes with food ad libitum

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Abstract. Age and size at metamorphosis were studied in two half-sibling *Salamandra infraimmaculata* larval cohorts born and raised in the laboratory under three different density regimes, and fed ad libitum until they metamorphosed. There was no significant effect of density on the number of larvae metamorphosing. In the two cohorts studied, a significant positive relationship was observed with age at metamorphosis that increased with density, and a significant negative relationship between mass and density. In both cohorts, density did not appear to have an effect on either minimal or maximal age, mass or length at metamorphosis, nor on the range between maximum and minimum. There was no significant difference between the two cohorts in either age or length at metamorphosis. The difference in mass increased with density. The evolutionary significance of density effects on size (mass, length) and age at metamorphosis under unlimited resource (food) conditions, is by spreading out emergence of post-metamorphs onto land and their subsequent dispersal, and by affecting their size as adults and thereby their eventual maturity.

Key words. Caudata, larval cohorts, density effects, metamorphosis.

Introduction

Urodeles exhibit plasticity in the timing of, and in achieving an optimal size at metamorphosis (WILBUR & COLLINS 1973, WARBURG 2011). The duration of the larval period (i.e., their age or the time to metamorphosis), as well as their size at metamorphosis depend on their larval growth history (discussed in COHEN et al. 2005, 2006). Both the timing of, and the size at, metamorphosis are critical for survival of the young post-metamorphs and as a consequence, for the recruitment of juveniles into the terrestrial population (WARBURG 2011).

This is especially true when conditions are suboptimal as are those prevailing on Mt. Carmel, Israel, during the breeding season of *Salamandra infraimmaculata* MARTENS, 1885. Winter rains may start in October, eliciting breeding in the salamanders. In some years, rainfalls may be sufficient to fill the rock pools, which are their main breeding sites. However, in about 50% of the time, the pools dry out, resulting in the loss of entire larval cohorts (WARBURG 2009a). Nevertheless, when rains eventually resume, some larvae may be saved, since they can survive for about a week on wet mud (WARBURG 1986a,b).

The question remains: why start breeding early in the rainy season when about half of the broods might be lost? The advantage in starting larval life as early as possible in

the season, lies in the advantage these larvae have when rainfall is continuous, since they are able to grow more rapidly by feeding on newborn larvae (COHEN et al. 2005, WARBURG 1992, 2009a, 2010). The larvae require at least five weeks to undergo metamorphosis (WARBURG 2011). If breeding is delayed because of delayed rainfall, there is a risk that time could be insufficient to allow metamorphosis (WARBURG 2010, 2011).

Aquatic fauna is rather poor when the ponds first form early in the season. Larvae then depend almost entirely on their cannibalistic (sibling predation) trait, preying on late-born larval cohorts in order to obtain enough food to facilitate their own growth (COHEN et al. 2005). The survival of larvae in spite of all these risks will enable their successful survival as post-metamorphs.

In addition, the emergence on land of the young post-metamorphs will have to be timed (i.e., actual date) so that the soil will still be moist and temperatures sufficiently low to make successful dispersal possible (WARBURG 2011). This depends on the amount of rains falling until then, and on the season. Emergence in winter is preferable to spring, which is characterized by hot spells (sciroccos). Moreover, both the larvae's age at metamorphosis (measured from their birth in the laboratory because it can rarely be recorded in nature), as well as the duration of the larval period (i.e., the time it takes to metamorphosis), are of great

significance. As a rule, larval period is positively related to age at metamorphosis. Finally, the size attained by the larvae when metamorphosing is of great consequence for the survival of the post-metamorphs and later of the adults. However, metamorphosis can be delayed due to slow larval growth rates, resulting in small size at metamorphosis. To conclude: successful survival to adulthood depends on starting life as a larva as early in the season as made possible, by rainfall (WARBURG 1992, 2010), growing rapidly (COHEN et al. 2006), avoiding cannibalistic predation by half-siblings (COHEN et al. 2005), metamorphosing as early in the season as possible and at an early age (WARBURG 2011), attaining a greater size, and, finally, dispersing as early in the season as possible in order to escape dehydration of the soil.

Density (i.e., larval crowding) was shown to have an effect on urodelan larvae with regard to both growth rate and development to metamorphosis (BRUNKOW & COLLINS 1996). OHDAKI (1994) has shown that density was negatively related to both time and size at metamorphosis. High density can cause failure in metamorphosis (WILBUR 1976). SCOTT (1990) in *Ambystoma opacum* (GRAVENHORST, 1807), and BRODMAN (1996) have shown in both *Ambystoma maculatum* (SHAW, 1802) and *A. jeffersonianum* (GREEN, 1827) that both size and larval period are negatively affected by density.

The objective of the present study is to establish to which extent three different density regimes affect both age and size (mass, length) at metamorphosis of half-sibling salamander larval cohorts born in the laboratory to the same female (i.e., a cohort), and fed ad libitum.

Materials and methods

The data discussed here are based on a long-term study (1974–1998) of a single breeding population of *Salamandra infraimmaculata* (WARBURG 2006, 2007a). This species is a 'rare' and endangered species found in the northern, mountainous part of Israel (IUCN Red List status "Near Threatened"; www.iucnredlist.org). The metapopulation inhabiting Mt. Carmel survives in the most southeastern portion of the genus' distribution.

Gravid females collected in the field delivered their larvae in the laboratory and were thereafter released into the wild (WARBURG 2007b, 2008, 2009a). This is an ovoviparous species, laying eggs that hatch upon contact with water (WARBURG et al. 1978/79). During the study period, a total of 74 half-sibling larval cohorts were born in the laboratory (WARBURG 2009c, 2010), two of which were used for the study described in this paper. Thus, the identity of their mothers, their dates of birth, and their sizes at birth were all known. These cohorts contained 4,085 larvae, all born to these freshly collected females. Most of the larvae were released into the ponds where their mothers had been collected. Of the remaining larvae, 396 were raised in the laboratory (at room temperature) until they metamorphosed (COHEN et al. 2005, 2006). Others were raised for an additional

two to five years after metamorphosis in order to release them into the wild as juveniles (WARBURG unpubl. data).

Larvae were placed in glass 'finger bowls' (13.5 cm in diameter) or enamel troughs (15 × 25 cm) filled with stale (aged) tap water, 2 cm in depth. They were fed ad libitum (i.e., the larvae had available unlimited amounts of food until they completed metamorphosis) with live *Tubifex* worms or minced beef liver (no significant differences were found in the effects of these two types of food on either age or size at metamorphosis). The *Tubifex* were added after cleaning the troughs and were always available to larvae. The water was changed every day.

Larval salamanders usually stay on the bottom most of the time, hiding among stones. Only towards the end of the metamorphic cycle will they swim to the surface for air. Consequently, density is calculated per surface area rather than volume. Larvae were placed either individually into finger bowls (42.4 cm²/larva), or in enamel troughs in groups of either five ('low density', 28.7 cm²/larva) or 10 larvae ('high density', 14.3 cm²/larva). Three replicates of each constellation were used. Towards the end of their larval period, they were transferred into spacious plastic containers with both water and soil that enabled them to crawl onto land and metamorphose.

As the sample size was rather small, I used both t-tests and regression analysis for statistical analyses with Sigma-Plot 9.0. The relationships between age, mass, and length, and the three density conditions were tested using regression analysis. The difference between the two cohorts was examined by t-tests.

Results

The data for means and ranges in age and dimensions (mass, length) of the two half-sibling larval cohorts are given in Tables 1 and 2. Cohort I consisted of 96 larvae, and cohort II of 106 larvae. There was no significant difference between the two cohorts (Appendix 1). No significant effect of densities on the numbers of emerging metamorphs could be seen in either cohort (Figs. 1, 2) (Appendix 2). A significant positive relationship between increasing age and density was noted in cohort I (Figs. 3A–C) (Appendix 3). Likewise in cohort II, age at metamorphosis related significantly to densities as did mass and length (Fig. 4A–C). In both cohorts, no significant effect of density could be demonstrated as affecting either mass or length with age (Figs. 5A–C, 6 A–C) (Appendix 4). Density did not appear to have an effect on either minimal or maximal age except at high densities in cohort II. In both cohorts, minimal and maximal mass and length did not show any significant difference (Fig. 7 A–C). The range between minimal and maximal age at metamorphosis showed an insignificant positive relationship with increasing density in both cohorts (Fig. 8 A), whereas both mass and length at metamorphosis decreased with density. Finally, there was no significant difference in either age, mass or length between metamorphosing larvae belonging to the two cohorts (Fig. 9 A–C) (Appendix 1).

Metamorphosis of *Salamandra infraimmaculata*

Table 1. Age, mass, and length at metamorphosis of specimens in cohort I, fed ad libitum and raised under different density regimes (single: larvae kept individually in glass jars (42.4 cm²/larva); low = larvae kept at a density of 28.7 cm²/larva; high = larvae kept at a density of 14.3 cm²/larva). Means are followed by standard deviation, the range in parentheses and the sample size (N).

	single	low	high
age (days)	44.1 ± 2.04 (41–47); N = 6	45.45 ± 2.56 (40–51); N = 6	46 ± 3.28 (41–76); N = 6
mass (g)	1.63 ± 0.14 (1.46–1.6); N = 16	1.36 ± 0.22 (1.03–1.79); N = 16	1.29 ± 0.23 (1.07–1.55); N = 16
length (mm)	66.3 ± 3.76 (64–70); N = 6	60.1 ± 1.87 (57–64); N = 16	60.2 ± 3.16 (56–70); N = 10

Table 2. Age, mass, and length at metamorphosis of specimens in cohort II, fed ad libitum and raised under different density regimes. For abbreviations see Table 1.

	single	low	high
age (days)	44.4 ± 2.07 (61–70); N = 10	44.6 ± 4.28 (40–51); N = 9	47.9 ± 7.91 (41–76); N = 16
mass (g)	1.69 ± 0.09 (1.52–1.83); N = 10	1.51 ± 0.23 (1.24–1.92); N = 9	1.44 ± 0.21 (1.08–1.97); N = 16
length (mm)	65.6 ± 2.95 (61–70); N = 11	61.8 ± 3.68 (57–68); N = 9	59.2 ± 3.54 (54–65); N = 16

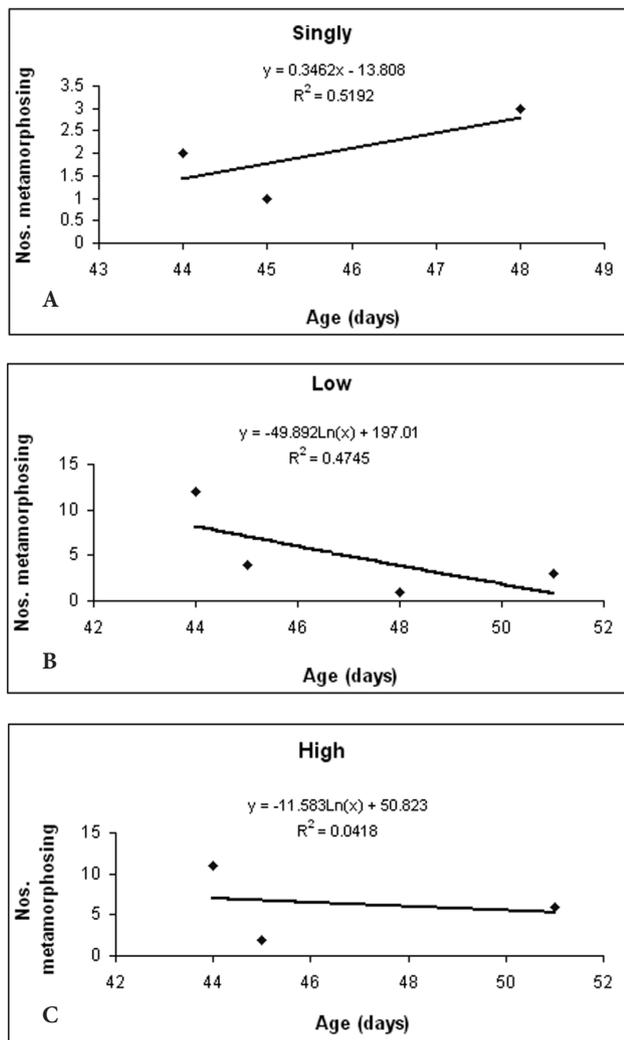


Figure 1. Density effects on numbers of metamorphs in cohort I. (A = single, B = low, C = high). See Appendix 2.

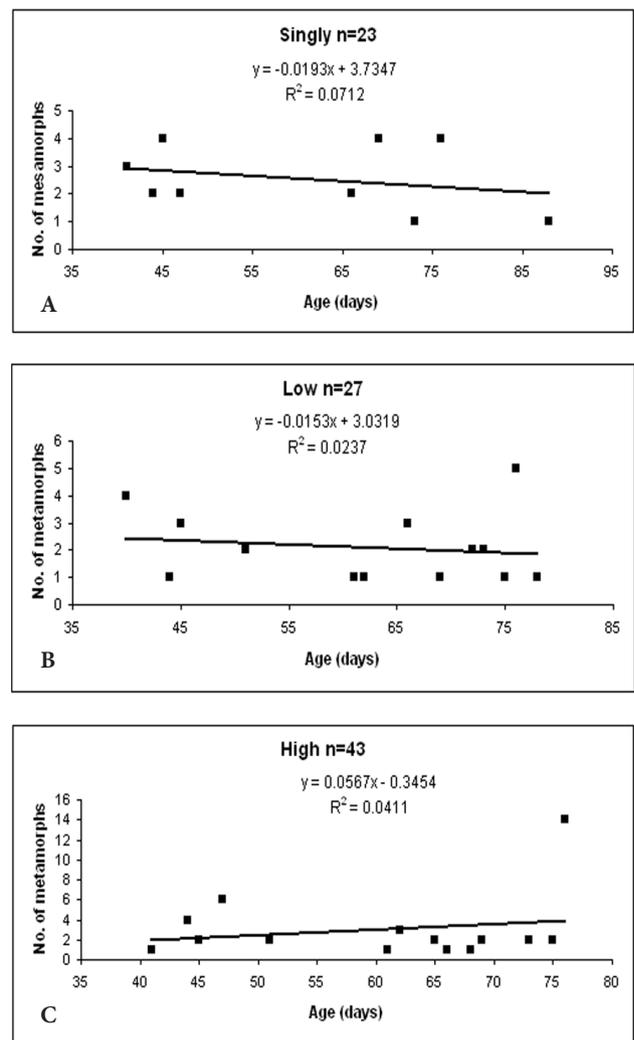


Figure 2. Density effects on numbers of metamorphs in cohort II. (A = single, B = low, C = high). See Appendix 2.

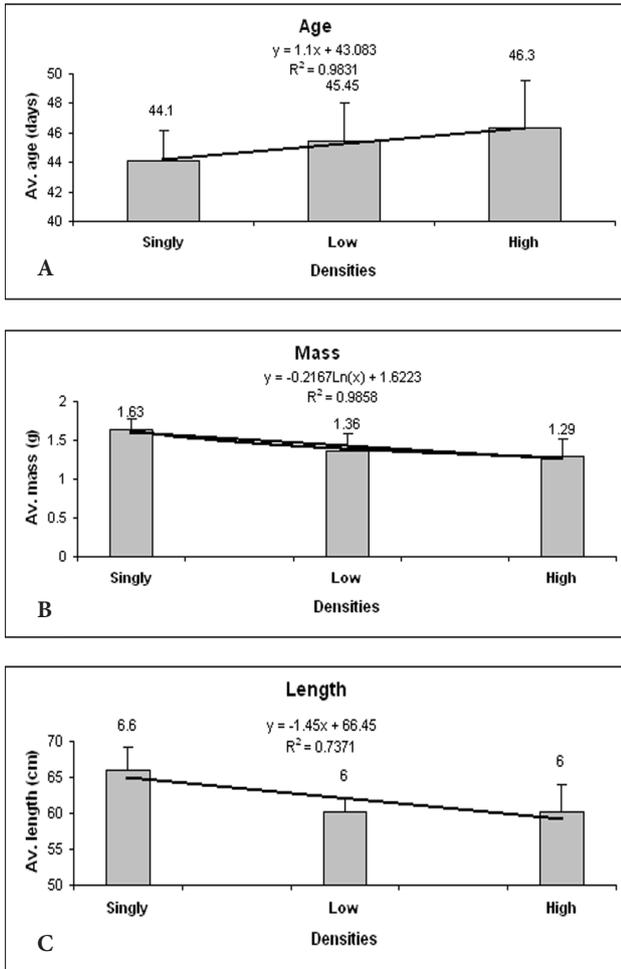


Figure 3. Average (A) age, (B) mass, and (C) length at metamorphosis in cohort I when raised under three different densities. See Appendix 3.

Discussion

The main problem with studies on aquatic amphibian larvae concerns their origin and age, both of which are generally unknown. Thus, in some studies on density effects on metamorphosis in urodeles, neither age nor larval period could be known with accuracy since the larvae studied had been collected in the field (CHAZAL et al. 1994). In others, individual egg clutches in nests attended by females were collected in the field, separated, and hatched in the laboratory. Consequently, although the larval period was known (since the eggs hatched in the laboratory) in such cases, it was nevertheless not identical with age (from the actual point of time when the eggs had been laid) (KUSANO 1981, COLLINS & CHEEK 1983, FAUTH, RESETARITS & WILBUR 1990, DOODY 1996, NISHIHARA 1996, WALLS 1998). Moreover, in neither scenario can it be ascertained whether the larvae were indeed half-siblings, because it cannot be ruled out that either the larvae or the batches of eggs indeed originated from more than one female. In some other studies,

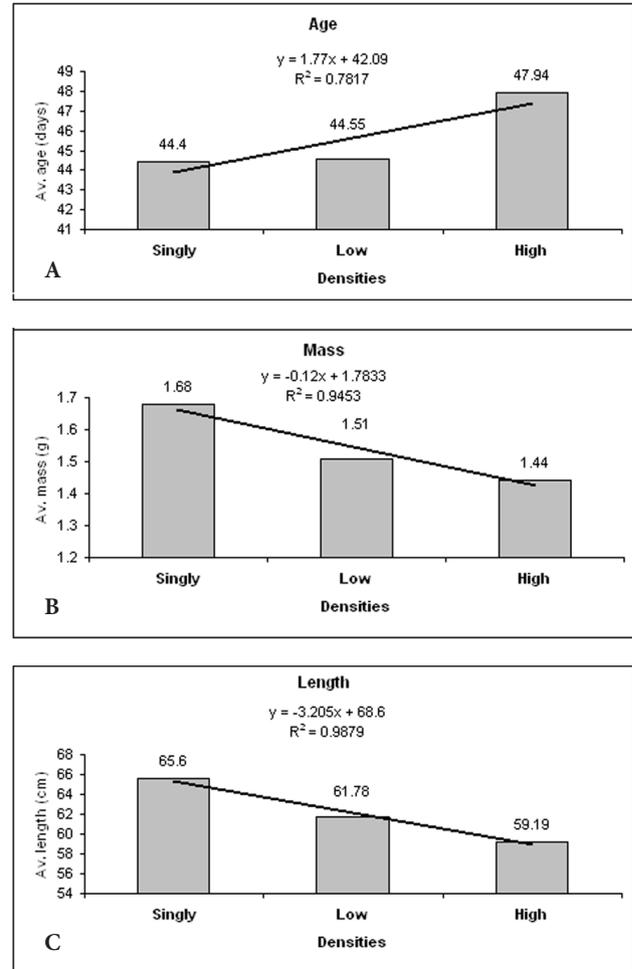


Figure 4. Average (A) age, (B) mass, and (C) length at metamorphosis in cohort II when raised under three different densities. See Appendix 3.

both age and larval period were known since individual clutches of half-siblings were either collected (DOODY 1996), or adult females laid eggs that hatched in the laboratory (ZIEMBA & COLLINS 1999, COHEN et al. 2005, 2006).

Some of the variability found in the period to metamorphosis and size of post-metamorphs can be attributed to these facts. Thus, it is not surprising that WILBUR (1976) noticed a high variance in both time and size at metamorphosis within the same pond. Variation in duration of larval period and body size at metamorphosis was attributed to time of oviposition and density of conspecific larvae. Such individual variation can affect population dynamics (BRUNKOW & COLLINS 1996).

Most studies attempted to show the effects of density (i.e., crowding or sharing the space with one or more larvae), and resource (i.e., food resources) on time required until metamorphosis and size of post-metamorphs. Few studies tried to isolate and analyse these factors separately: the effect of density when food is available at all times, or the effect of food when larvae are raised singly without

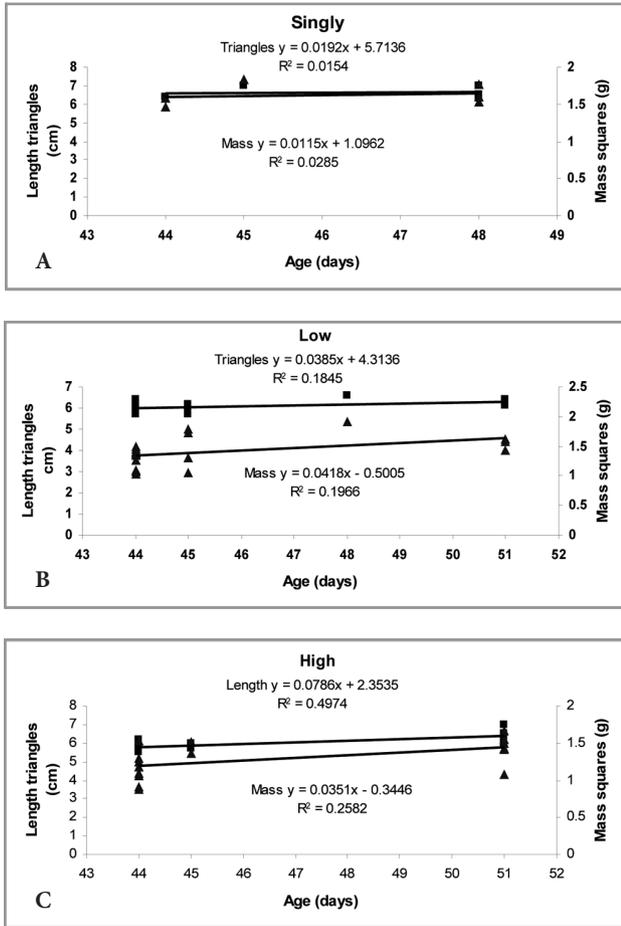


Figure 5. Age, mass, and length at metamorphosis in cohort I (A: single, B: low density, C: high density). See Appendix 4.

any potential density effect (BRODMAN 1999, ALCOBENDAS et al. 2004, WARBURG 2009b, 2010). In the last mentioned study it was concluded that metamorphosis appears to be affected by food resources when density could not have been a factor since these larvae were raised individually. Table 3 summarizes density conditions in six different studies of larvae belonging to four different urodelan species that were raised on an unlimited food supply. The conditions vary greatly between different experiments. Thus, low-density conditions ranged from 16.5 cm² per larva of *Hynobius retardatus* (DUNN, 1923) (see WAKAHARA 1995) to 502.7 cm² per larva of *Ambystoma tigrinum nebulosum* HALLOWELL, 1853 (COLLINS & CHEEK 1983): a 30.5-fold difference. Likewise, high-density conditions ranged from 75 cm²/larva of *Salamandra salamandra* (LINNAEUS, 1758) (DEGANI 1993) to 42.7 cm²/larva of *Ambystoma texanum* (MATTHES, 1855) (PETRANKA 1984): a 5.7-fold difference. In the present study, *S. infraimmaculata* larvae were raised at a low density of 28.7 cm²/larva or a high density of 14.3 cm²/larva. Since experimental conditions vary so much between different studies on the significance of density in urodelan larval growth and metamorphosis, it is diffi-

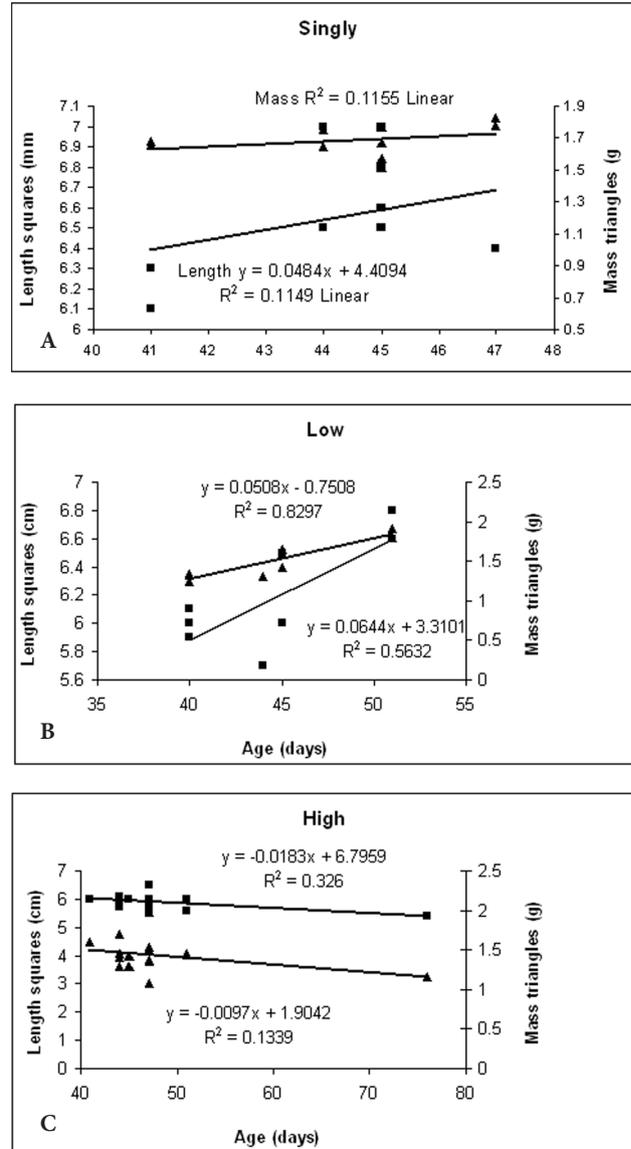


Figure 6. Age, mass, and length at metamorphosis in cohort II (A = single, B = low density, C = high density). See Appendix 4.

cult to compare them. Thus, the high density of 42.7 cm² in *A. texanum* larvae (PETRANKA 1984), is close to a moderate density of 43.7 cm² in *S. salamandra* larvae (DEGANI 1993). Raising larvae of *A. maculatum* at high densities significantly affected the number of metamorphs (WALLS 1998). Generally, urodelan larvae raised under high-density conditions took longer to metamorphose and did so at a smaller size. It was shown that when *A. opacum* larvae were raised under high-density conditions, which results in smaller post-metamorphs, growth and first reproduction were delayed by three years (TAYLOR & SCOTT 1997).

In the present study, it was shown that although neither competition nor sibling-predation (i.e., cannibalism) were possible (as potential cannibal larvae were removed once identified as such), and food was constantly available, den-

Table 3. Density (cm² per larva) data from the literature for larvae of different salamander species raised on an unlimited food supply (ad libitum). Total numbers of larvae are given in parentheses. In order of appearance data were taken from: DEGANI (1993), REQUES & TEJEDO (1996), COHEN et al. (2006), COLLINS & CHEEK (1983), PETRANKA (1984), WAKAHARA (1995).

Species	low density	medium density	high density	food type
<i>Salamandra salamandra</i>	75 (20)	43.7–75 (20)	7.5 (20)	aquatic invertebrates
<i>Salamandra salamandra</i>	300 (2)			pond zooplankton
<i>Salamandra salamandra</i>	28.7 (4–5)		14.3 (9–10)	<i>Tubifex</i> and minced liver
<i>Ambystoma tigrinum nebulosum</i>	502.7 (3)	215.4 (7)		<i>Artemia</i> nauplii
<i>Ambystoma texanum</i>	342 (1)	85.5 (4)	42.7 (8)	aquatic invertebrates
<i>Hynobius retardatus</i>	16.5–33 (5)	13.2–16.5 (20)	8.25–11 (40)	urodelan larvae

sity affected both age and size at metamorphosis. This supports hypothesis 2 of PETRANKA & SIH (1986), which states that larval growth was density-dependent because of ‘interference competition’, a hypothesis that did not find support in their own study on *A. texanum* larvae. A similar

situation existed in the experiment conducted by EITAM et al. (2005). The true meaning of this term needs yet to be clarified. It is the behavioural aspects of density effects that are largely unknown. What could be the exact nature of such interference? This term could comprise a number of

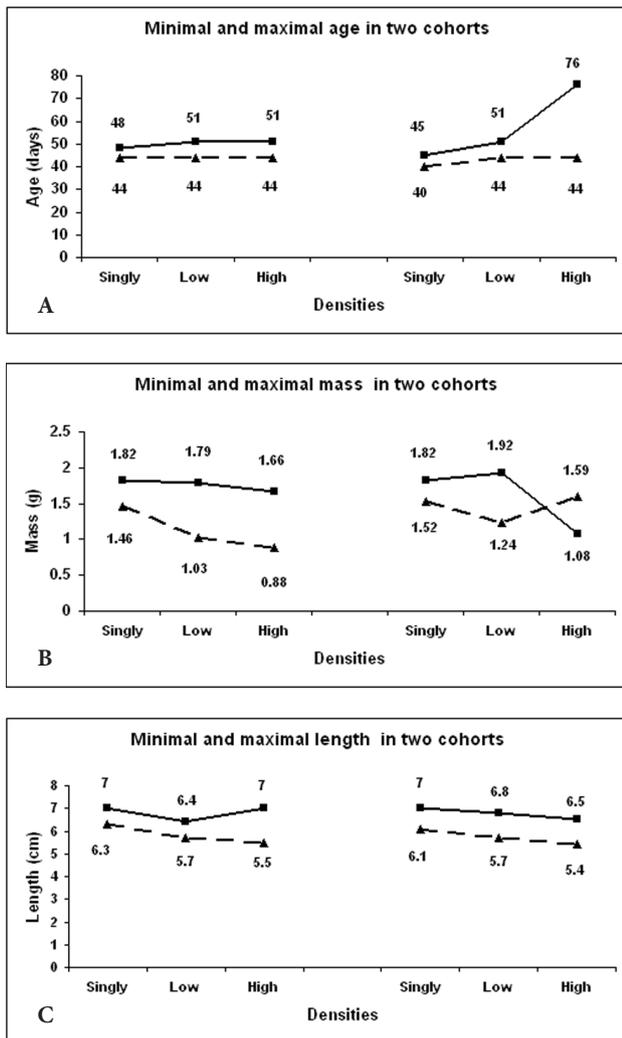


Figure 7. Minimum and maximum (A) age, (B) mass, and (C) length at metamorphosis in the two cohorts.

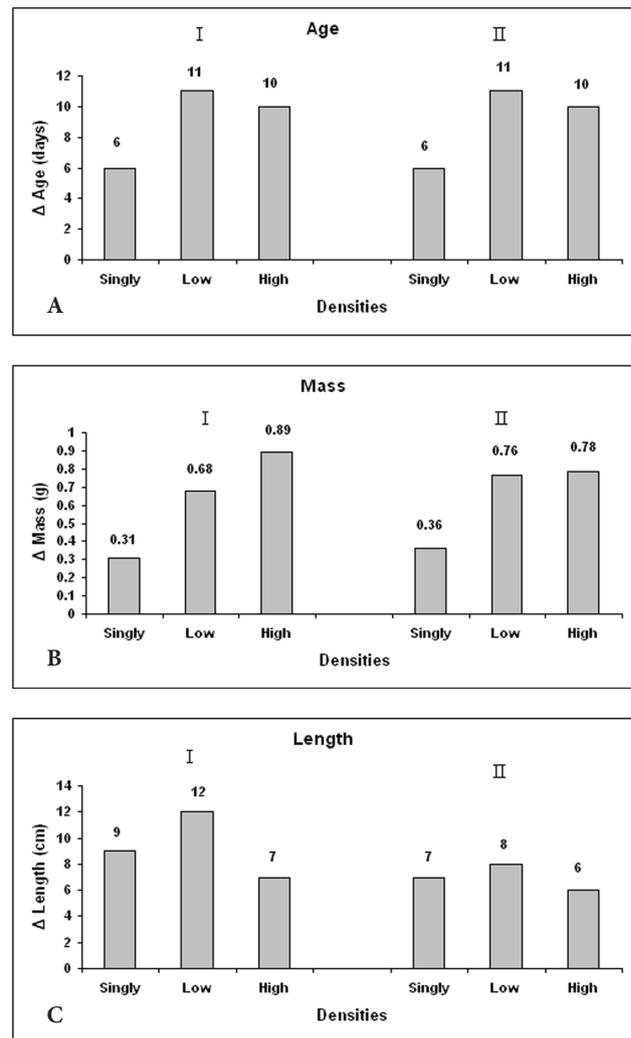


Figure 8. Range between minimal and maximal (A) age, (B) mass, and (C) length at metamorphosis in the two cohorts.

Table 4. Differences between low and high density ($\Delta\%$) in number, age, and mass at metamorphosis when larvae of different salamander species at low and high densities were raised and fed ad libitum.

species	number of larvae ($\Delta\%$)	age ($\Delta\%$)	mass ($\Delta\%$)	source
<i>Ambystoma opacum</i>	981 (81.7 %)	2.6 %	47.3 %	SCOTT (1990)
<i>Ambystoma opacum</i>		38.8 %	49.2 %	SCOTT (1994)
<i>Salamandra infraimmaculata</i>	5 (50 %)	4.4 %	4.9 %	this study

factors: (1) Since food (especially live food) is generally not evenly distributed, it is possible that some larvae are quicker than others in locating it. These will have an advantage as they may keep others from feeding. (2) It is possible that a sensory response (optical, olfactory or tactile) is involved that is sufficiently deterrent to keep some larvae from feeding regularly. Thus, it is theoretically possible that in spite of the fact that food was constantly available, some larvae fed less or less frequently than others.

In *A. opacum*, density affected both larval period (i.e., age at metamorphosis), which varied from 2.6 to 38.8%, and mass at metamorphosis, which differed by about 48% when raised at low and high densities (SCOTT 1990, 1994).

In *S. infraimmaculata*, the differences between both age and mass when raised at low and high densities were 4.4 and 4.9% respectively (Table 4).

Differential growth rates of larvae have previously been demonstrated in this species (COHEN et al. 2006). These were due to different growth modes identified within a single half-sibling larval cohort and resulted in variable growth rates. Consequently, the variability in the age of larvae at metamorphosis could range from two to four months. Such wide variability could have catastrophic effects on the timing of metamorphosis and subsequent juvenile dispersal. In this harsh Mediterranean climate, it makes all the difference whether larvae metamorphose during winter rather than spring, which can well be too hot and dry for the post-metamorphs to survive.

What could be the cause of the definite effect of density (larvae raised in the company of other larvae) under seemingly food resource-independent conditions that was demonstrated here? On the face of it, food could not possibly be the cause of this effect since it was always freely available, but perhaps the presence of other larvae already deters some from feeding ad libitum? This point is of great interest and definitely needs further investigations. The evolutionary significance of these findings could be in showing the way how larvae are capable of spreading out the timing of metamorphosis (i.e., age at metamorphosis), and thereby spreading out post-metamorphic growth, which would result in spreading out the age at maturation. This would mean that the aquatic larval population were capable of regulating the size of the adult terrestrial population. Keeping the larval population low in small ponds under unpredictable hydroperiod conditions would improve chances of survival in species living under unpredictable ambient conditions (WARBURG 2007b).

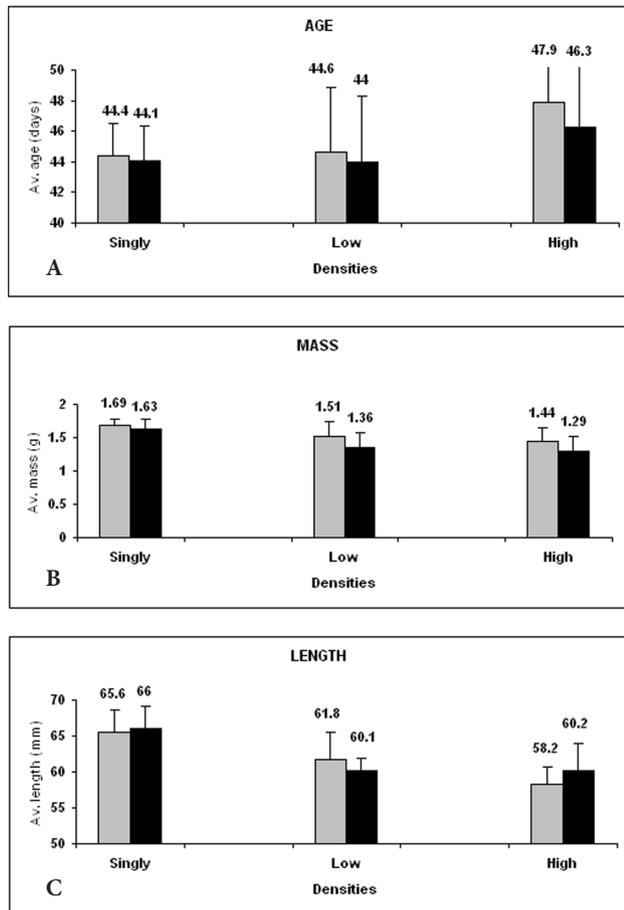


Figure 9. Average of (A) age, (B) mass, and (C) length at metamorphosis in cohorts I and II. See Appendix 1.

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Metamorphosis of *Salamandra infraimmaculata*

Appendix 1

Results of t-tests of data on cohort I versus those on cohort II.

Age	single	low density	high density
T value	0.330005	0.231763	0
P value	0.745007	0.819489	1
degree of freedom	19	17	30

Mass	single	low density	high density
T value	0.981452	1.650592	1.889448
P value	0.343027	0.111849	0.068216
degree of freedom	14	24	31

Length	single	low density	high density
T value	-0.25577	1.543785	-0.87974
P value	0.801845	0.135725	0.385772
degree of freedom	14	24	31

Appendix 2

Results of regression analysis of age under different density regimes in relation to Figures 1 and 2.

Cohort I	R ²	P	degree of freedom
single versus age	0.9313	0.531	3
low density versus age	0.7767	0.2889	4
high density versus age	0.757	0.806	3

Cohort II	R ²	P	degree of freedom
single versus age	0.8401	0.0598	9
low density versus age	0.7281	0.7432	13
high density versus age	0.4973	0.8015	14

Appendix 3

Results of regression analysis of age under different density regimes in relation to Figures 3 and 4.

Cohort I: single	age	mass	length
R ²	0.9941	0.9981	0.9981
P	0.5193	0.1829	0.1829
degree of freedom	6	6	6

Cohort I: low density

	age	mass	length
R ²	0.9779	0.9989	0.9996
P	0.5879	0.0001	< 0.0001
degree of freedom	20	20	20

Cohort I: high density

	age	mass	length
R ²	0.9779	0.9982	0.9989
P	0.6382	0.0261	< 0.0001
degree of freedom	17	17	17

Cohort II: single

	age	mass	length
R ²	0.9963	0.095	2
P	0.9993	< 0.0001	2
degree of freedom	0.9986	0.0198	2

Cohort II: low density

	age	mass	length
R ²	0.983	0.9984	1
P	< 0.0001	< 0.0001	0
degree of freedom	16	16	16

Cohort II: high density

	age	mass	length
R ²	0.9974	0.9984	0.9982
P	0.1882	0.0703	0.0085
degree of freedom	10	10	10

Appendix 4

Results of regression analysis of age under different density regimes in relation to Figures 5 and 6.

Cohort I

	R ²	P	degree of freedom
age versus mass	0.9941	0.5193	6
age versus length	0.9981	0.1829	6
mass versus length	1	0.06	6

Cohort II

	R ²	P	degree of freedom
age versus mass	0.9974	0.1882	10
age versus length	0.9984	0.0703	10
mass versus length	1	0	10