Age and size at metamorphosis of half-sib larvae of *Salamandra infraimmaculata* born in the laboratory and raised singly under three different food regimes

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ABSTRACT. *Salamandra infraimmaculata* is an endangered amphibian urodele species inhabiting an unpredictable xeric environment at the edge of its zoogeographic distribution on Mt. Carmel, Israel. This area is characterized by early October rains of short duration forming rock pools where this salamander breeds. Since these shallow ponds dry out rather soon, the larvae have limited time to develop and metamorphose. It is of great importance that they reach this stage of development quickly and attain the largest size possible.

This study examined metamorphosis under experimental conditions using half-sib larvae of the same age raised singly under different food regimes without any stress of density.

It was found that age, mass and length at metamorphosis regressed significantly with food regimes when salamander larvae were raised singly. Thus, a significant difference in age and mass at metamorphosis was found between larvae raised singly on three food regimes. Age was least, and both mass and length were greatest when larvae were fed '*ad-libitum'*.

Minimal and maximal age at metamorphosis increased significantly and mass decreased significantly as food became scarcer.

Consequently, metamorphosis appears to be affected by food resources when density was not a factor. The significance of this finding is discussed.

KEY WORDS : *Salamandra,* Caudata*,* Larval cohorts, Resource effects, Metamorphosis

INTRODUCTION

Several risks are involved in the successful completion of metamorphosis in nature for individuals of the species *Salamandra infraimmaculata* Martens, 1855:

1. Will rain start early and in sufficient quantities to fill the ponds, with sufficient water to sustain larval growth until completion of metamorphosis? The break in rain should be short so that the ponds will not dry out, allowing larvae born early to survive (WARBURG, 1992).

2. Will there be enough food to enable growth (COHEN et al., 2006) of early-born larvae? Will it be enough so that late-born larval cohorts, that may provide food for early-born cannibal larvae (COHEN et al., 2005); will also be able to survive?

3. Will larval density be low enough to enable a short development period and a large size at metamorphois?

The successful survival of post-metamorphs depends on the survival of the larvae in spite of all these risks.

Both age at metamorphosis (measured from their birth in the laboratory), as well as the duration of the larval period (i.e. the time it takes to metamorphose), are of great significance. Moreover, the size attained by the larvae until metamorphosing is of great consequence to the survival of the post-metamorphs. These questions have been addressed in several previous studies on urodeles (WILBUR & COLLINS, 1973). However, the two main factors important to metamorphosis, density and resources (i.e. food), have rarely been analyzed separately except by LICHT (1992) who raised solitary larvae of *Ambystoma gracile,* and both OHDACHI (1994) and KOHMATSU et al. (2001) who raised solitary larvae of *Hynobius retardatus)* to metamorphosis.

In the present study an attempt is made to study the effect of food resources when half-sib larvae were raised singly from birth, with no other larvae competing or preying thus ruling out the density effect. The effect of density on metamorphosis when food is available *ad-libitum*, will be examined separately.

MATERIALS AND METHODS

The study is based on long-term (1974-1998) observations on a single breeding population of *Salamandra infraimmaculata* (WARBURG, 2006; 2007a,). This salamander is a rare and endangered species found in the northern part of Mt.Carmel, Israel.

Gravid females were collected near the breeding ponds and allowed to release their larvae in the laboratory. This salamander species is ovoviviparous, laying eggs containing completely formed larvae wrapped in an egg envelope. The larvae hatch immediately upon contact with water (WARBURG et al., 1978/79). The females were then released back to nature (WARBURG, 2007 b; 2008). During the study period a total of 74 cohorts of half-sib larvae were born in the laboratory. Thus their mothers and the larvae's dates of birth are both known. These cohorts contained 4085 larvae all born to these freshly-collected females. Most of the larvae were released back to the ponds where their mothers were collected, when one day old (see WARBURG, 2009a). Of the remaining larvae, 396 were raised in the laboratory (at room temperature) until they metamorphosed (COHEN et al., 2005; 2006), while others were raised for an additional 2-5 years after metamorphosis to be released as juveniles back to nature (in preparation).

The larvae were placed singly into 'finger bowls' (13.5cm diameter, 42.4cm^2/larva). Since salamander larvae usually stay on the bottom most of the time, surfacing only to gulp air towards the end of the metamorphic cycle, the calculation is per area rather than per volume. The finger bowls were filled with aged tap water 2cm in depth that was changed daily after feeding.

Larvae received 0.02g minced liver (wet weight) per larva, or 0.01g live *Tubifex* larvae per larva (plenty of food), 0.005g liver per larva or 0.004g live *Tubifex* larvae per larva (little food) and the last group received an unlimited amount of live *Tubifex* larvae during the experiment ('*ad-libitum*'). No significant differences have been found in the effects of these two food items on either size or time to metamorphosis (COHEN et al., 2005). At the age of 18 days the amount of *Tubifex* provided was quintupled and at the age of 23 days the amount of chopped liver was doubled. For 'little' and 'plenty' food conditions, larvae were fed every 3-4 days and the bowls were cleaned of food leftovers 1-1.5hrs after feeding, and filled with fresh water. For '*ad-libitum*' conditions live *Tubifex* larvae were added after cleaning and were always available.

Towards the end of their larval period soil was added enabling the larvae to crawl out and metamorphose.

The data were analysed in several different ways.

1. The means and the standard deviation of each data series were calculated. The latter provided a method by which the variability within the data can be evaluated. It is well known that comparing means is not sufficient since the same mean can be obtained with different data series. The difference between the means of age, mass and length at metamorphosis under three different food regimes was examined by t-tests between the means.

2. In each data series minimum and maximum values as well as the range between them were used as different parameters. These values are to a certain extent related but not necessarily so. Thus, the same range between minimum and maximum can be observed in different data series with different minimum and maximum values. In other words range alone is not sufficient to evaluate data.

3. To compare between two data series I have used regression analyses. Thus, the relationships between age, mass and length were tested using regression analysis.

RESULTS

Age and mass at metamorphosis were studied in a number of half-sib larval cohorts (Fig. 1). The difference in the average age and mass between the different cohorts was not significant. Age, mass and length at metamorphosis regressed significantly with food regimes (R^2=0.9188, R^2=0.9942 and R^2=0.9442 linearly) (Fig. 2 A-C). Likewise, there was a marked difference in both age and length at metamorphosis when larvae were raised on unlimited food (Fig. 2 A; C). Both mass and length increased significantly with age when larvae were raised on 'little' food (R^2=0.829 linear, and R^2=0.824 linear, respectively) but no significant relationship was noticeable when they were fed 'plenty' or *'ad-libitum'*. There was a significant difference (p=0.017) in mass between larvae raised with 'plenty' of food or '*ad-libitum*', and the difference in length from receiving '*ad-libitum*' or 'little' food, was highly significant ($p<0.0001$).

The relationship between age, mass and length is shown for individual metamorphs in Fig. 3. The variability among individuals is remarkable. Likewise, the number of larvae metamorphosing varied greatly (Fig. 4). Both inter- and intra-cohort differences can be seen.

Both minimal and maximal age at metamorphosis regressed significantly (Fig. 5 A). Age at metamorphosis increased as food became scarcer (R^2=0.9734 logarithmic, and R^2=0.9235 logarithmic, respectively). Similarly, both minimal and maximal mass at metamorphosis regressed significantly with food regimes (Fig. 5 B). Thus, metamorphs became smaller with food becoming scarcer (minimal mass R^2=0.9981 linear, and maximal mass R^2=0.9169 logarithmic). There was a significant difference in mass among the three food regimes. On the other hand, metamorphs were not significantly shorter when food became scarce (Fig. 5 C).

The range between minimal and maximal age, mass and length decreased significantly when food became scarce (Fig. 6 A-C). Thus, range in age decreased significantly when food became scarce (R^2=0.9959 linear, Fig. 6 A), likewise in mass (R^2=0.9346), and in length (R^2=0.7797) both logarithmic, Fig. 6 B; C).

The differences in age at metamorphosis (60%) between larvae raised on 'little' food or *ad-libitum* exceeded the differences in mass (34%) and length (43.7%).

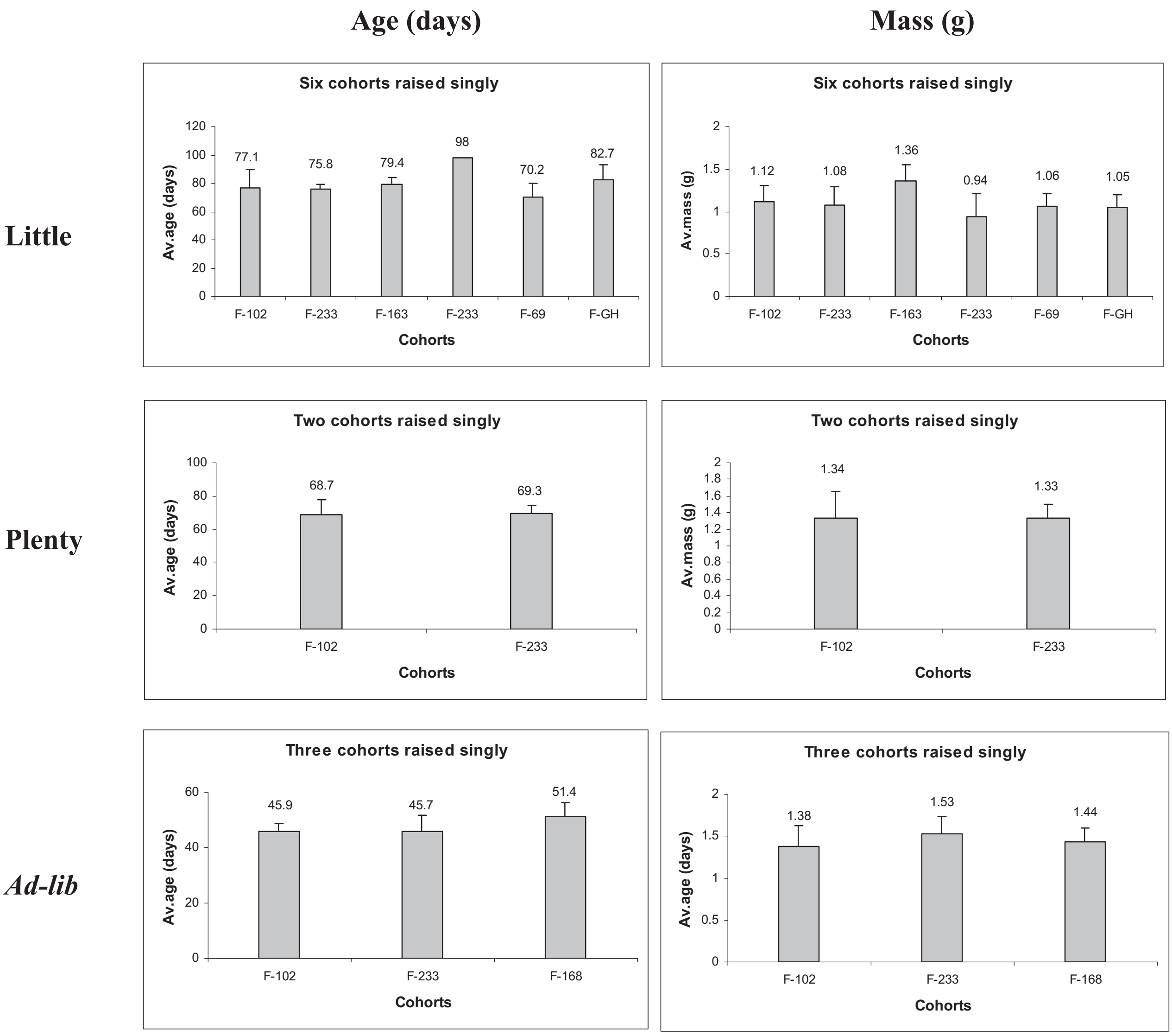

Fig. 1. – Average age and mass at metamorphosis of half-sib larval cohorts raised singly under different food regimes.

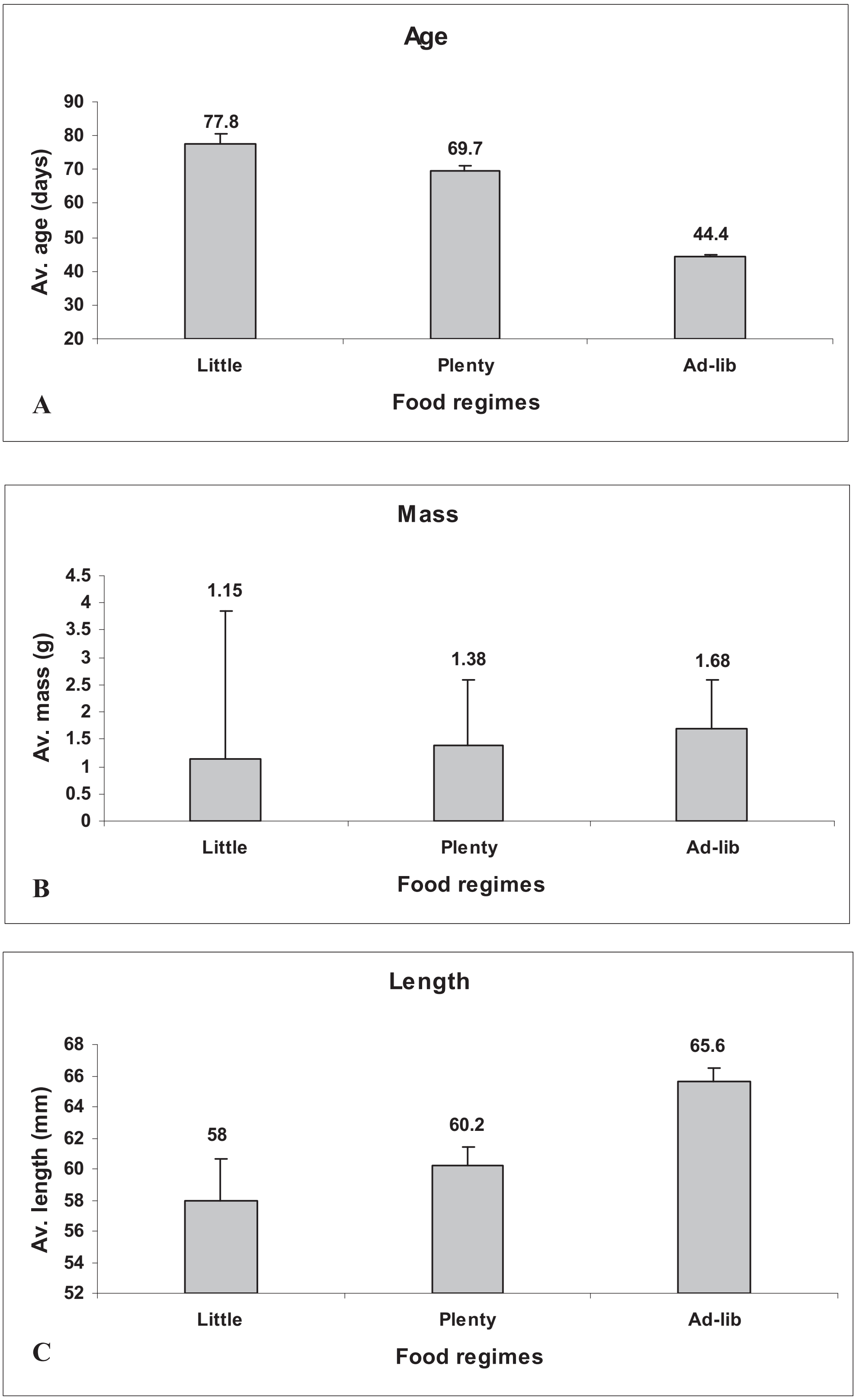

Fig. 2. – Average age, mass and length at metamorphosis when half-sib larvae belonging to a single cohort, were raised singly under three different food regimes.

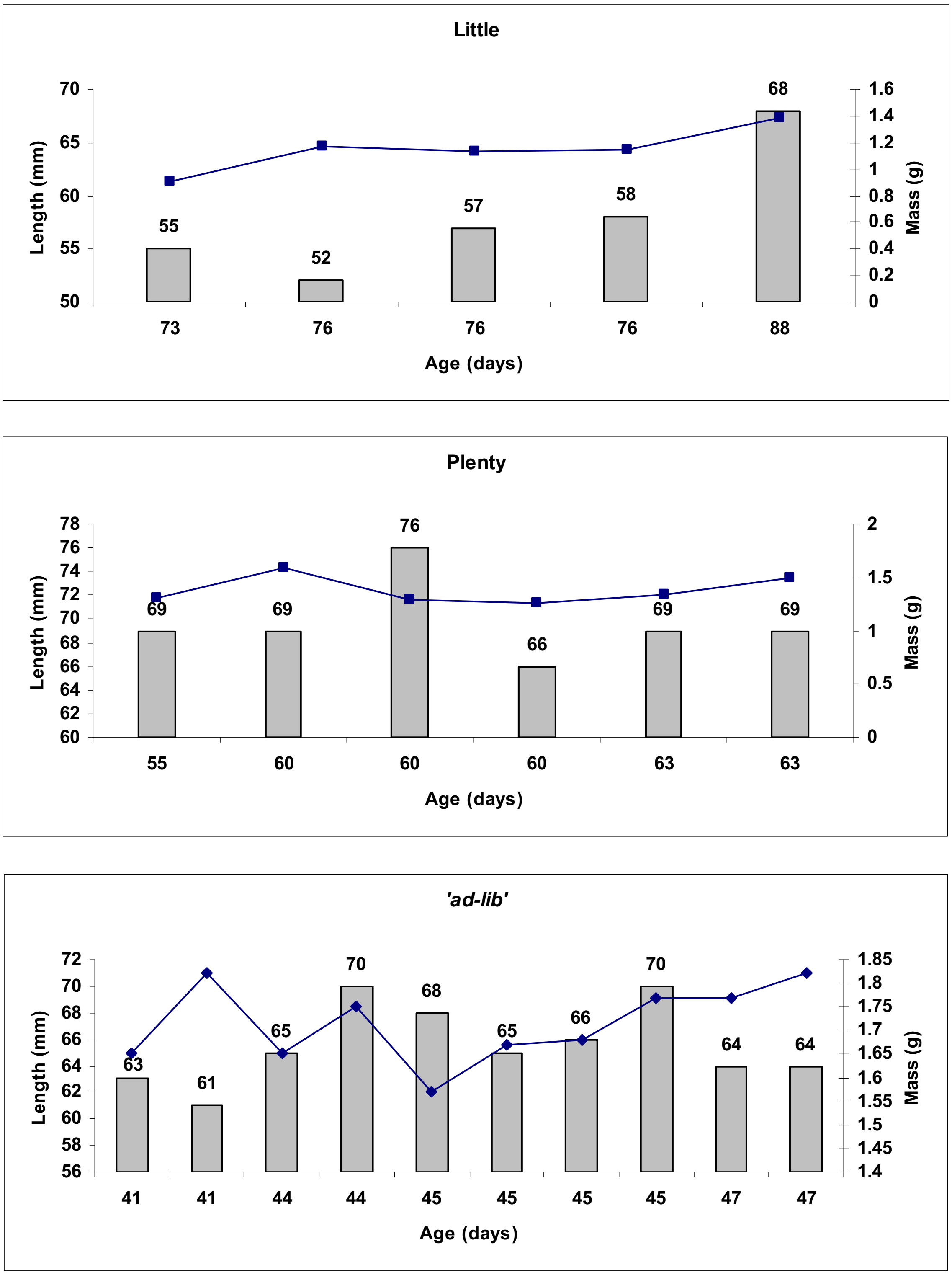

Fig. 3. – Relationship mass/age (line), and relationship length/age (column) at metamorphosis of half-sib larvae raised singly under different food regimes.

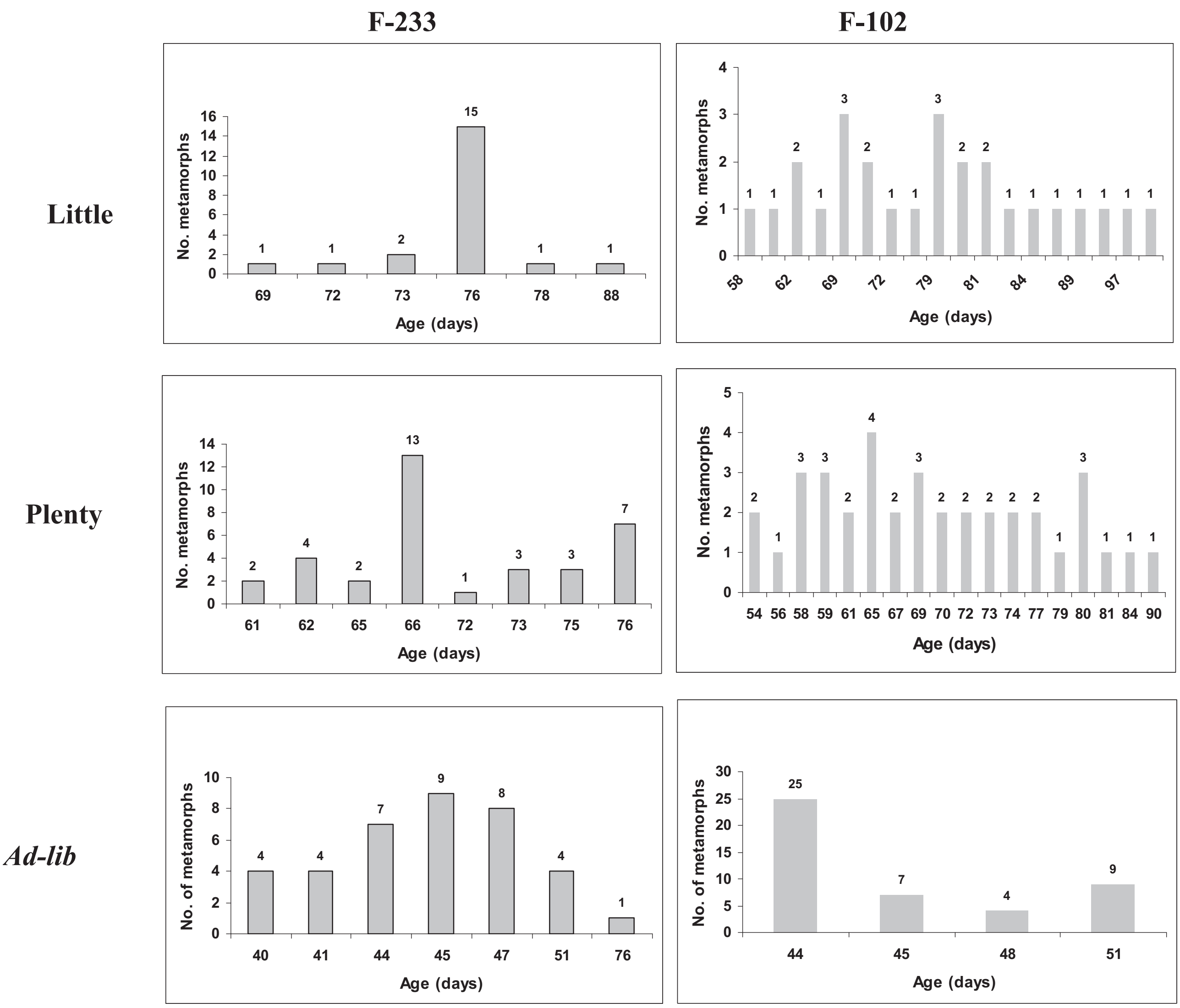

Fig. 4. – Number of metamorphs in two cohorts born on the same day and raised singly under different food regimes.

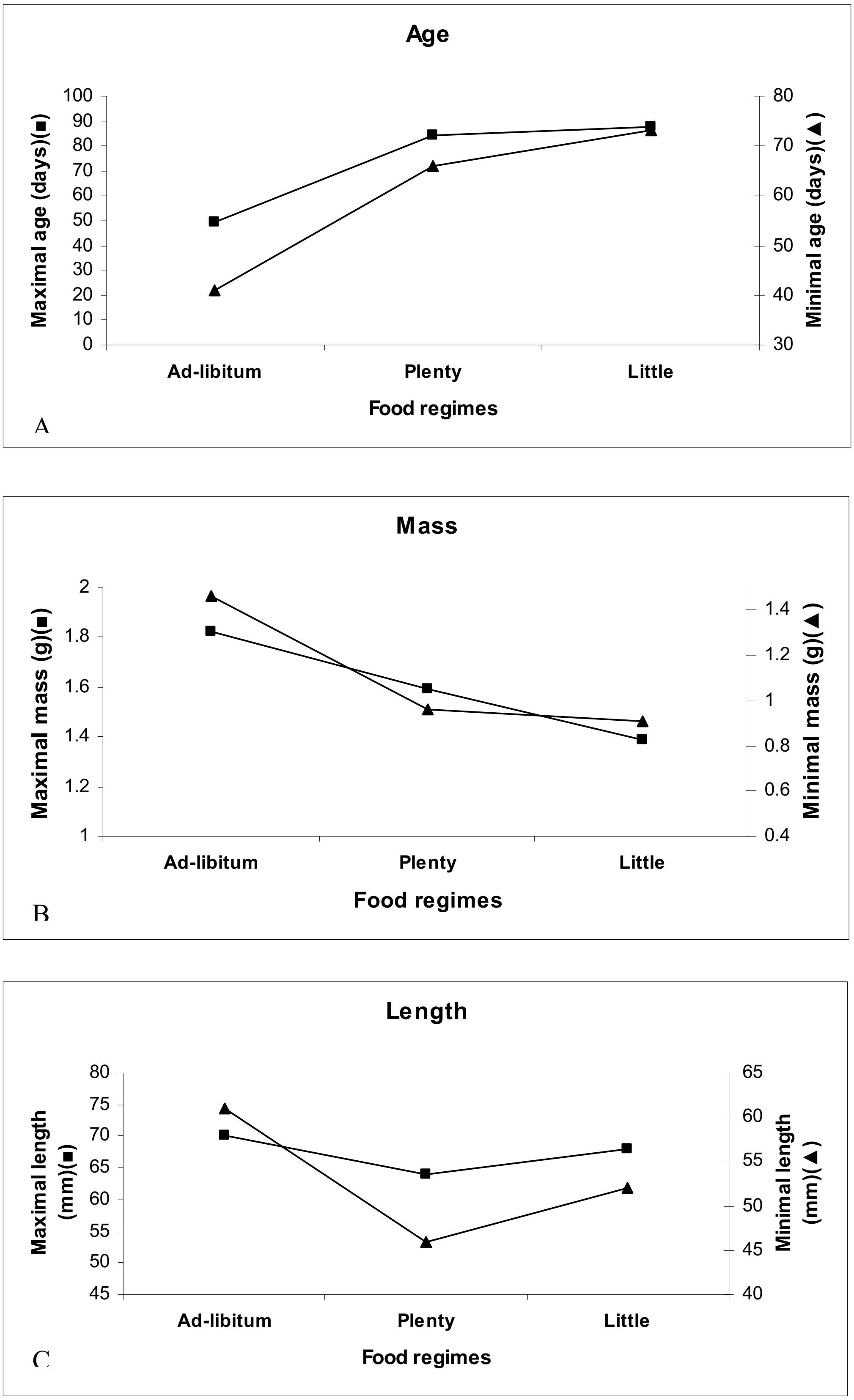

Fig. 5. – Maximal and minimal age, mass and length at metamorphosis in half-sib larvae raised singly under three different food regimes.

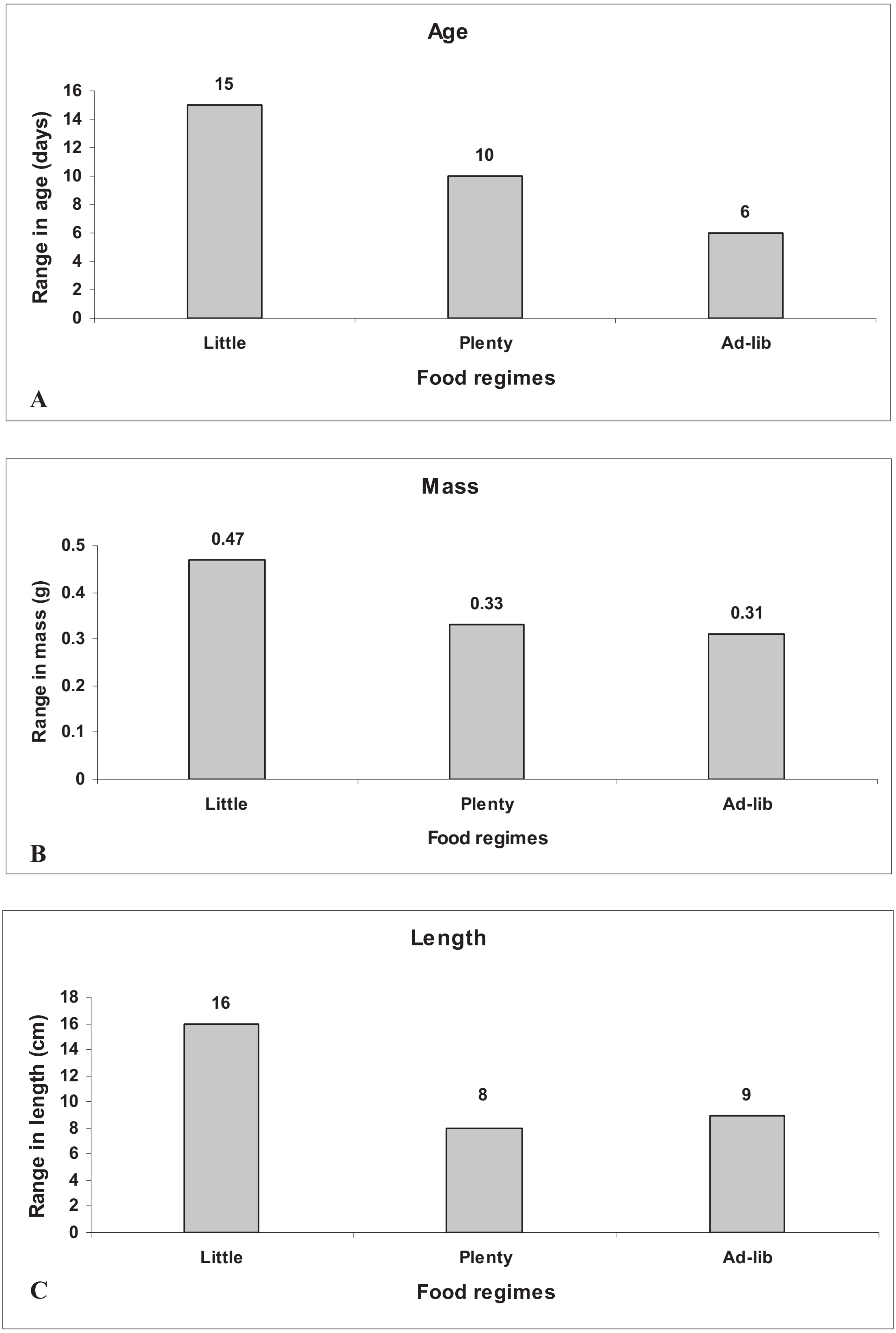

Fig. 6. – Range between maximal and minimal age, mass and length at metamorphosis.

DISCUSSION

Larval period and age of metamorphs

These are two different parameters: larval period is the time from when larvae are born or, alternatively, hatched from eggs laid. In the first case larval period will actually be their age, whereas in the second, larval period needs to be supplemented by the time it takes from the egg being laid to the time the larva hatches.

In some studies on this subject conducted on urodeles, age at metamorphosis was indeed known. Thus, *S. s. terrestris* females deposited their larvae in individual containers guaranteeing that larvae were half-siblings (Kopp & Baur, 2000). In other studies, age can not be known with certainty since either larvae (Licht, 1992; Brunkow & Collins, 1996; Hickerson et al., 2005) or eggs (in *H. retardatus* Ohdachi, 1994, in *Desmognathus ochrophaeus* Beachy, 1995, in *Ambystoma maculatum* Walls, 1998, and in *H. retardatus* Kohmatsu et al., 2001) were collected in the field and hatched in the laboratory. Consequently, although the larval period is known, it is not identical with age, which remains unknown since the time eggs have been laid is not known. In both of these cases it can not be accurately known that the larvae are half-sibs since the egg batches may belong to more than a single female (communal egg-laying).

The effects of density and food resources on larval growth and thus on metamorphs

Most studies attempted to show effects of density (i.e. crowding) and resource (i.e. food) on time to metamorphosis and size of post-metamorphs.

Hickerson et al., (2005) showed that metamorphic timing or the larval period in *Desmognathus quadramaculatus* was affected by food. In the same way, high food levels accelerated metamorphosis in *D. ochrophaeus* (Beachy, 1995) and in *Hemidactylium scutatum* (O'Laughlin & Harris, 2000).

Variations in *H. retardatus* larval growth affected both size and larval period (Kohmatsu et al., 2001). However, different food regimes under which larvae of *A. gracile* were raised did not differ in their effects on variation in mass at metamorphosis (Licht, 1992). When larvae were raised on low and high food levels no significant differences could be detected in body mass at metamorphosiss between those raised with food continuously available (‘*ad-libitum*’) and those fed on low food levels (Licht, 1992). Similarly, no effect on size at metamorphosis was observed in *Ambystoma tigrinum* (Bizer, 1978) nor did food regimes have any significant effect on days to metamorphosis in *D. quadramaculatus* (Hickerson et al., 2005). In larvae of *Ambystoma opacum* raised on a high food level, a significant correlation was noted with size at metamorphosis (Scott & Fore, 1995).

The effect of food when larvae are raised singly with no density effect possible was studied in *A. tigrinum nebulosum* raised in individual containers on three food levels: high, medium and low (Collin & Cheek, 1983). A similar study by Kohmatsu et al. (2001) on larvae of *H. retardatus* raised in solitude found no significant difference in size or days to metamorphosis.

Maximum and minimum, ranges and variations

Metamorphosis is not temporally fixed in time nor is it allometrically fixed in dimensions of metamorphs. There are larvae that take longer to metamorphose and attain smaller dimensions than others. Wilbur (1976) noticed very high variance in the same pond. Thus, variation in duration of larval period and body size at metamorphosis was attributed to density of conspecific larvae and time of oviposition. O'Laughlin & Harris (2000) raised 120 larvae of *H. scutatum* under different feeding regimes studying mass at metamorphosis and larval period. Different food regimes caused some differences in larval periods. They noticed 4.6% difference in age and 26.7% differences in mass at metamorphosis. Such important individual variation can affect the adult population.

In the present study it was shown that both age and size of salamander larvae at metamorphosis is resource (food) dependant. In addition, marked temporal and allometric differences in metamorphs were noticed when larvae were raised under each food regime. Differences of over 30% were found in both age (32%) and mass (31.5%) at metamorphosis.

What can be the cause of such significant variations in age and size at metamorphosis? Since this variation is present in all experimental setups, the explanation may be that these half-sib larvae have a multi-paternal origin.

Multiple mating and sperm storage are both known in *Salamandra* and therefore sperm mixing may take place (discussed in Warburg, 2009b). It was previously shown that there is variability in mass of new-born larvae within a half-sib cohort ranging between 5.1-10.1% (Cohen et al., 2005). This can be an outcome of multiple mating resulting in multi-parenthood. Since salamanders are known to be capable of storing sperm in the dorsal roof of the cloacal gland, the spermathecae, it is possible that certain sperm-mixing does take place in the uterus. As for sperm-storage, the fact that females arrive near the ponds in consecutive years (Warburg, 2006) rules out the need to store sperm in their spermathecae since a salamander gets more than a single chance to mate and can mate every year.

This variability increased during larval growth because larvae used different growth modes (Cohen et al., 2006). Consequently, variation in dimensions more than tripled by the time these larvae metamorphosed.

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