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The Evolutionary Significance of Intra-cohort Cannibalism in Larvae of a Xeric-inhabiting Salamander: An Inter-cohort Comparison

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Abstract: Cannibalistic behaviour in seven *Salamandra salamandra inframaculata* half-sib cohort larvae (each born to a single female and at the same time) and in juveniles was studied under different food and density conditions. The level of cannibalistic behaviour (tails bitten off or larvae eaten) changed as the larvae grew, from a low level during the first week to a peak at five weeks, regardless of differences in mass between the experimental larvae. No cannibalistic behaviour was observed in post-metamorphic salamanders even if they were cannibalistic as larvae. Significant differences in levels of cannibalism were found among different cohorts. Whereas in one cohort only 7% of the larvae were cannibalistic, in another, the cannibalism level peaked at 70% thereby indicating a possible maternal effect. However, cannibalistic behaviour in salamander larvae was apparently not related to the mother's age. The effect of food and density on cannibalism seems to be indirect or of secondary importance. Thus, cannibalism level was similar when food was scarce or when fed ad-libitum, whereas larvae offered a high level of food were significantly more cannibalistic. This could indicate that an optimum food level triggers cannibalism. When food becomes abundant the need for cannibalism ceased to persist. Under xeric conditions, ponds dry out rapidly; consequently rapid development through cannibalism results in earlier metamorphosis essential for this species' survival because of the limited time for dispersal of juveniles.

Key words: Cannibalism; Density and food effects; Metamorphosis; *Salamandra* larvae; Sperm mixing; Unpredictable climate

INTRODUCTION

The rare ovoviparous salamander, *Salamandra salamandra inframaculata*, inhabits a xeric habitat in a fringe region in northern Israel within the south-easternmost limit of its Palearctic distribution (Degani and Warburg,

1978). In this habitat on Mt. Carmel winter rainfall is infrequent, unpredictable, and varies greatly from year to year, creating extremely difficult conditions for an amphibian. Rain ponds contain water only for a short period, thereby limiting the time available for completion of metamorphosis (Warburg, 1992, 1997). Consequently the timing of the onset of breeding is crucial since larvae need at least five weeks to achieve metamorphosis. The winter

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rainfall ponds in this habitat are very poor in invertebrate life at first, and therefore food is scarce (Warburg et al., 1978/79). This increases natural cannibalistic behaviour among the salamander larvae in these ponds (Degani et al., 1980). Later, additional salamander larval cohorts are added to the food chain, thereby enhancing cannibalistic predation by the older and more developed larvae (Warburg, 1992).

The pre-metamorphic period (first 3–4 weeks) of amphibian larvae is entirely dedicated to growth (Etkin, 1963), and is followed by differentiation during the pro-metamorphic period (at 5–6 weeks). In that period, the three main organs involved in terrestrial adaptation of amphibians (skin, lungs and kidneys) differentiate and become functional with terrestrial breathing and excretion at metamorphic climax (Warburg, 1997; Gealekman and Warburg, 2000). Under these developmental circumstances, it is advantageous for larvae to be cannibalistic; thereby enhancing growth and reaching the pro-metamorphic period sooner.

Numerous studies have suggested different factors that affect cannibalistic behaviour including social factors (Elgar and Crespi, 1992), kinship (Waldman, 1991; Pfennig and Collins, 1993; Pfennig et al., 1994; Pfennig, 1997; Wakahara, 1997), larval, maternal, and paternal cues (Blaustein and O'Hara, 1982; Hoffman and Pfennig, 1999), body size (Walls and Semlitsch, 1991; Reques and Tejedo, 1996), and density and food level (Degani et al., 1980; Collins and Cheek, 1983; Semlitsch and Reichling, 1989; Crump, 1992, review; Degani, 1993; Wakahara, 1995; Reques and Tejedo, 1996). The conclusions from these studies are not uniform since some suggested that food level had no effect on cannibalistic behaviour (Collins and Cheek, 1983; Semlitsch and Reichling, 1989), whereas others suggested that cannibalism takes place solely in the absence of other food (Crump, 1992; Wakahara, 1995). The conclusions regarding the effect of density on cannibalism are even more confused. While some have found a negative correlation between density and cannibalism (Semlitsch and Reichling, 1989), others have

found positive correlation (Collins and Cheek, 1983; Crump, 1992; Wakahara, 1995; Reques and Tejedo, 1996). In most of these studies less cannibalism toward siblings than toward non-siblings was observed, although there are some exceptions (Walls and Blaustein, 1995). Therefore, kin recognition, cues of larval, maternal/paternal origin, and social factors all appear to have some effect on cannibalistic behaviour.

In the present study we aimed at clarifying the effects of different food levels and density conditions on larval cannibalism in the ovoviparous salamander *S. s. infraimmaculata*. All the larvae used belonged to the same cohort (i.e. born in the laboratory on the same day to one mother that was freshly collected from the field). Moreover, the study compared intra-cohort cannibalistic behaviour among different cohorts of this salamander (i.e. cannibalism within a certain cohort in comparison to that in another cohort originating from a different female). Since in this xeric habitat only a few females breed in these rock pools that are poor in invertebrate life at the beginning of the season, the larvae depend entirely on other larvae as a food source (i.e. sib predation or cannibalism). In view of the great significance of cannibalism in maintaining populations of this xeric-inhabiting salamander under extreme and unpredictable climatic conditions, we decided to undertake a study on this subject aiming at clarifying two main points: 1. Does cannibalism change as larvae grow and metamorphose? 2. Are there differences in cannibalism among different cohorts? Thus, we followed the ontogenesis of cannibalistic behaviour through larval growth and after metamorphosis, under different food and density conditions and compared this behaviour among different cohorts.

MATERIALS AND METHODS

Larvae

Seven gravid *S. s. infraimmaculata* females (A–G) were collected on Mt. Carmel, Israel, during the breeding seasons (October–January)

TABLE 1. Experimental set-up of the present study. Letters A–G refer to seven females of *Salamandra salamandra inframaculata*, from which larval cohorts for experiments were obtained.

Treatment	Number of replicates per female							Larvae per Replicate	Total no. of larvae per treatment	Total no. of replicates
	A	B	C	D	E	F	G			
High density										
adlibitum	0	0	2	0	0	2	0	10	40	4
abundant food	0	2	2	1	2	2	2	9–10	108	11
scarce food	0	2	2	1	1	2	2	9–10	96	10
Low density										
ad-libitum	0	0	2	0	0	3	0	5	25	5
abundant food	2	2	2	2	2	3	0	4–5	64	13
scarce food	2	2	2	2	2	3	0	4–5	63	13
Total no. of larvae per female	20	60	85	40	49	105	37		396	56

TABLE 2. Cannibalism in post-metamorphic individuals of *Salamandra salamandra inframaculata*. Cohorts from females A, D, and E were used. ΔW (%), percentage of differences in mass within larval pairs; N, no. of replicates.

ΔW (%)	n			Cannibals (%)
	Inter-cohort	Intra-cohort	Total	
0–9	10	11	21	0
10–39	10	11	21	0
40–100	4	2	6	0
100–150	2	1	3	0

of 1996–1998. This subspecies is very rare. Therefore, the experimental set-up was limited by the number of breeding females during the short breeding season, besides the number of larvae per cohort. Since only a few females breed every year during a limited breeding season (Warburg, 1992, 1994), and only some of them produce a sufficiently large cohort of larvae, this study spread over four years. These ovoviviparous females spawned sibling cohorts of live larvae in separate bowls in the laboratory (see Warburg et al., 1978/79), and their development was followed from birth to metamorphosis. A total of 396 such larvae born in the laboratory from seven different

cohorts were studied (see Table 1). The broods of females A, D, and E continued to be tested for cannibalistic behaviour after metamorphosis (Table 2).

Effects of density and food levels

For these experiments 396 larvae were placed in 56 glass bowls 13.5 cm in diameter, filled with aged tap water 2 cm in depth. Larvae were fed with measured amounts of live *Tubifex* worms or fine chopped beef liver (no significant differences were found in the effects of these two food items on either growth rate or time till metamorphosis). Larvae received 0.02 g fine chopped beef liver (wet weight) per larva, or alternatively 0.01 g live *Tubifex* per larva ('abundant food'), 0.005 g liver per larva or 0.004 g live *Tubifex* per larva ('scarce food') and the last group received an unlimited amount of *Tubifex* during the experiment ('ad-libitum'). At the age of 18 days the amount of *Tubifex* was quintupled (increased five-fold) and at the age of 23 days the amount of chopped liver was doubled. For scarce and abundant food conditions, larvae were fed every three to four days and the bowls were cleaned 1–1.5 hrs after feeding since by then no food remained, and filled with fresh water. For ad-libitum conditions *Tubifex* were added

after cleaning and were always available.

As the salamander larvae stay on the bottom most of the time, the density was calculated per area rather than per volume. Twenty-five bowls contained 10 larvae under high-density conditions (14.3 cm^2 per larva) from which larvae in eleven bowls received abundant food, larvae in ten bowls received scarce food and larvae in four bowls received food ad-libitum. Thirty-one bowls contained four to five larvae under low-density conditions (28.7 cm^2 per larva), in which larvae in thirteen bowls received abundant food, larvae in thirteen bowls received scarce food, and larvae in five bowls were fed ad-libitum (see Table 1).

All bowls containing the animals were kept at room temperature in the laboratory. Larvae of females A, B, D, E and G were fed with chopped beef liver and larvae of females C and F were fed with live *Tubifex* worms. We used two different food types in order to be able to compare our results to other studies where different food items were used. Due to the cannibalistic phenomenon, which characterizes larvae of this sub-species (Degani et al., 1980; Degani, 1993), the initial number of larvae declined during the experiment. Consequently, in order to maintain constant density conditions larvae were transferred into suitable-sized glass bowls: 13.5 cm in diameter for 9–10 larvae ($14.3\text{--}15.9 \text{ cm}^2$ per larva), 12 cm in diameter for six to eight larvae ($14.1\text{--}18.8 \text{ cm}^2$ per larva) and 9.5 cm in diameter for two to five larvae (less than $14.2\text{--}17.7 \text{ cm}^2$ per larva).

Cannibalism

We checked daily for evidence of bitten-off tails, which indicate extreme aggressiveness and a cannibalistic potential since tails of larvae were bitten gradually up to the vent, as a result of which the larva died. Percentage of bitten tails generally (except for female D cohort, $n=40$) correlated with that of cannibals ($r=0.821$, $P=0.045$), and therefore it can be used as an early indication for cannibalism level in a population. A larva was identified as

a cannibal when found with another larva in its mouth. Since the cannibalistic potential rather than its effect on growth and metamorphosis was studied, cannibals, once identified as such, were removed from the bowl and the remaining larvae transferred into suitably sized glass bowls. The percentage of cannibals among the larvae in the experiment was calculated and thus the level of cannibalism was determined.

Postmetamorphic salamanders

Juveniles or post-metamorphs, offspring of females A, D, and E, were used for this experiment when the post-metamorphs were three months old. Pairs of juvenile salamanders were placed in a plastic container for seven days with neither refugia nor food. They could be identified individually by their typical yellow spots on their back. One salamander in every pair was known to have been cannibalistic as a larva. Each salamander was used only once.

Intra-cohort cannibalism of post-metamorphs was tested during their first three weeks after emerging on land by pairing salamanders from the same cohort. (it was considered meaningless to extend this period since in nature all juveniles disperse immediately following metamorphosis). For this experiment 16 juveniles of female E, 12 juveniles of female A, and eight juveniles of female D were used (Table 2). Differences in mass within the pairs varied from 145% to zero.

Inter-cohort cannibalism was tested during weeks four to six following metamorphosis. For this experiment female E juveniles were paired with female A juveniles (nine pairs of A–E), female E juveniles were paired with female D juveniles (eleven pairs of E–D) and female D juveniles were paired with female A juveniles (five pairs of D–A). Differences in mass within these pairs varied from 100% to zero.

Statistical analyses

In order to test the hypothesis that food and density affect cannibalism repeated measures

ANOVA test and Newman-Keuls test were used. Reduced alpha of 0.025 was employed because each data set was analyzed twice. Percentage data were angularly transformed prior to running statistical tests. The same tests were used to examine the differences between cohorts and age groups. Because of the rather limited size of available data set, we adopted repeated measures ANOVA with one-way factor design and tested the statistical significance of the effect of each factor separately.

RESULTS

Cannibalistic behavioral changes with age

Cannibalism in salamander larvae changed during the first eight weeks of the larval stage, disappearing entirely in the post-metamorphic salamanders (Fig. 1). During the larval stages, there was a highly significant difference ($P < 0.0005$) in the level of cannibalism as the larvae grew from week to week. Cannibalism level in all larval cohorts was relatively low during the first three weeks, peaked during 4–6 weeks, and then dropped (Fig. 1A, B). Cannibalism level during the first three weeks was significantly lower ($P < 0.0001$) than during 4–6 weeks of age; similarly the cannibalism level during 7–8 weeks of age was significantly lower ($P < 0.005$) than during 4–6 weeks of age. After reaching the peak, at 4–6 weeks, the cannibalism level dropped. When comparing cannibalism level during the first three weeks to that at 7–8 weeks, the latter was significantly higher ($P = 0.044$). These changes in the cannibalism level were unrelated to mass differences between the larvae, because the mass differences throughout larval growth were continuous and almost linear, whereas the cannibalism level appeared to be hyperbolic (Fig. 1B).

Postmetamorphic cannibalism

No cannibalistic behaviour was observed when post-metamorphic salamanders were tested, either inter- or intra-cohortly, even if they differed in size up to 145%, regardless of

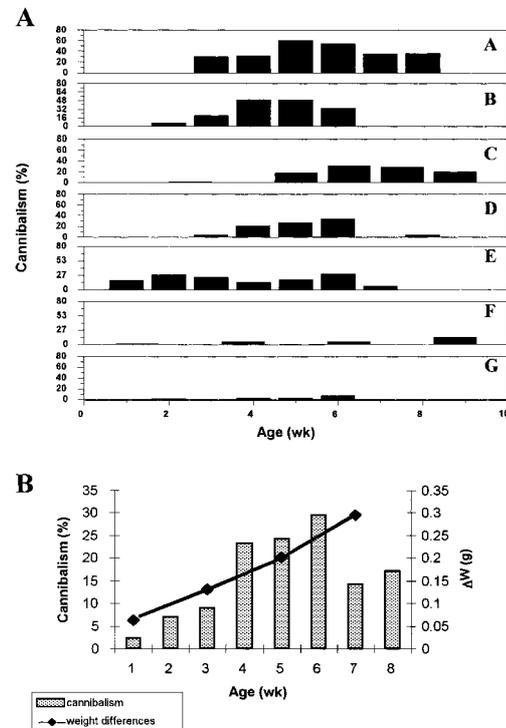


FIG. 1. Above (A): Ontogenesis of cannibalism in seven different cohorts (A–G). A similar pattern is noticeable in almost all cohorts, with exception of the larval cohort of female (F) that was monitored every other week. Below (B): Changes in cannibalism vs mass differences. Weight differences during larval growth are continuous whereas the ontogenesis of cannibalism level appears to follow a hyperbolic curve.

whether they were cannibalistic as larvae or not (Table 2).

Intra- and inter-cohort differences

The pattern of intra-cohort cannibalism was similar among larvae from different cohorts, but there was a significant difference in intra-cohort cannibalism level among the different cohorts (A–G) during the first five weeks (P ranging between < 0.0001 to < 0.05) (Fig. 1A). The larval cohort of female E showed cannibalistic behaviour from the first week and kept a constant level of 10–30% cannibals during the larval stage. Larvae of female F showed significantly ($P < 0.005$) less cannibalism in

TABLE 3. Cannibalism in larval *Salamandra salamandra infraimmaculata* belonging to seven different cohorts. Ages of females B–G were estimated on the basis of long-term observations at their original localities. For female A, age is unknown because of her different local origin.

Cohort	Bitten tails (%)	Cannibals (%)	Approximate age of female (yr)
A	90	25	unknown
B	61.6	16.6	21
C	32.9	2.6	4
D	57.5	0	12
E	68	20	20
F	24.2	0	18
G	8.1	10.8	19

comparison to the cohorts of other females. Larvae of female D presented a high aggression level (57.5% bitten tails), but no cannibals were identified (Table 3). The percentage of cannibalistic larvae in total differed between cohorts and seemed to be unrelated to the approximate age of the female (based on long-term observations on individual females by one of us (MRW, in preparation), as summarized in Table 3. Thus larval cohorts of similar aged females were highly variable in their cannibalistic behaviour.

When the larvae are born their weight and length vary very little (Warburg et al., 1978; Degani and Warburg, 1995). This can be seen from Table 4. The variability within a cohort can be as low as 5.1% of the average weight of the larvae in the cohort (or 5.1% of 310 mg which is a difference of 15.8 mg) or it can be as high as 10.1% of the average weight of the larvae (or 10.1% of 327 mg, which is a difference of 33 mg). The fact that there is variability at all can be due to the fact that these cohorts are of half-sibs and not full-sibs: thus, neither multi-paternity nor sperm mixing can be excluded (Sever, 1998, 2002; Rafinski and Osikowski, 2002). However, it has been shown that in xeric habitats the size of offspring at birth is more variable than in mesic habitats (Rebello and Leclair, 2003).

TABLE 4. Weights at birth ($\bar{x} \pm SD$) of larval *Salamandra salamandra infraimmaculata* from seven different females (A–G). Variability equals the proportion of SD against \bar{x} .

Female	Sample size	Weight at birth (mg)	Variability (%)
A	36	334.7 \pm 24.9	7.4
B	62	371.6 \pm 26.4	7.1
C	52	326.6 \pm 33.0	10.1
D	130	310.3 \pm 15.8	5.1
E	130	220.4 \pm 17.0	7.7
F	32	256.3 \pm 22.2	8.7
G	126	228.1 \pm 15.1	6.6

Effect of density

Due to the high variability in larval cannibalism during the first four weeks and again toward the end of the larval period, at 7–8 weeks of age, we decided to test the effects of food and density during the most variable time, at the age of five weeks (Fig. 1A). The effects of food and density were tested only during that period. No significant difference in cannibalism level was found among larval cohorts kept at high or low densities (Fig. 2A).

Effect of food

The effect of food on cannibalism level was likewise tested during the fifth week. There seemed to be no direct relationship between food level and cannibalism as illustrated in Fig. 2B. The differences in cannibalism level between low food and ad-libitum, under all density conditions were not significant ($P=0.3$, 0.15, and 0.07, respectively). However, the differences in cannibalism level between high food and ad-libitum under all density conditions were significant ($P<0.006$, <0.025 , and <0.0005 , respectively). Between high and low levels of food, the differences were significant only under low-density conditions and when looking at the total effect ($P<0.02$, $=0.4$, and <0.025 , respectively).

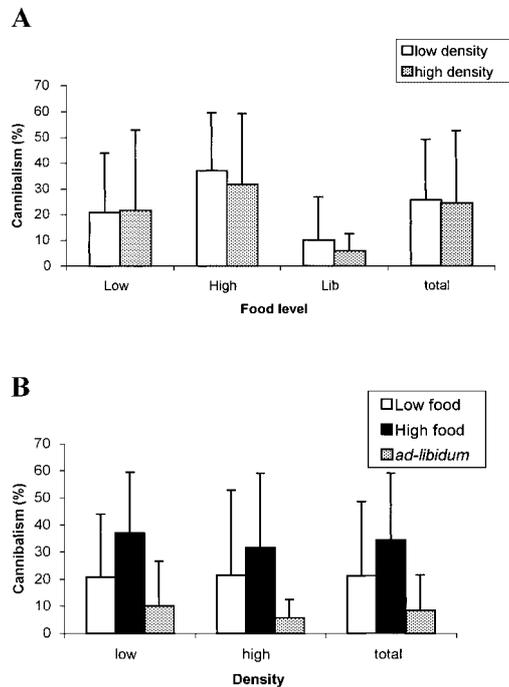


FIG. 2. Above (A): Effects of food and density on cannibalism at the age of five weeks. The effects of density at different food levels. The differences in cannibalism (%) between high and low densities are insignificant at all food levels. Below (B): The effects of food under different density conditions. The differences in cannibalism (%) between low food and ad-libitum are insignificant. The differences between high food level and ad-libitum are significant ($P < 0.006$, $P < 0.025$, $P < 0.0005$). The differences between high and low food levels are significant only under low-density conditions and when looking at the total effect ($P < 0.02$ and $P < 0.025$, respectively). Vertical bars indicate standard deviations (SD).

DISCUSSION

Evolutionary significance

The xeric-inhabiting salamander species studied here is exposed to xeric condition throughout the entire year (Warburg, 1994). Consequently it shows a series of adaptations (Warburg, 1997). One of these adaptations concerns its breeding in rock pools, which are the only water source available in the moun-

tains. The pools fill up late in autumn, when salamanders come to breed. Occasionally the ponds dry out within 2–3 weeks, due to an intermission in rainfall; this could cause the whole brood to perish (Warburg, 1992). Larva desiccation due to pond water holding duration can be a major component of reproductive failure in some years. There is a great advantage for cannibalistic larvae since the only food available at that time in the ponds are their conspecifics. Cannibalism as a form of intra-specific predation is the means by which a larva maximizes its growth and minimizes the time it spends in the pond (Wildy et al., 1998). It is sufficient to have one large cannibalistic larva metamorphosing in time to have a representative of that cohort, and two survivors to facilitate the survival of this species.

Some of the former experiments regarding cannibalistic and aggressive behaviours were conducted for short periods, e.g., minutes (Walls and Roudebush, 1991; Reques and Tejedo, 1996), whereas others were tested for longer periods e.g. one month or until metamorphosis (Degani et al., 1980; Collins and Cheek, 1983; Pfennig and Collins, 1993; Wakahara, 1995). Moreover, in some of the previous studies larvae were captured (Wildy et al., 1998) or eggs were collected (Ohdachi, 1994; Walls and Blaustein, 1995; Ziemba et al., 2000; Kohmatsu et al., 2001; Michmae and Wakahara, 2001, 2002a, b) and assumed to be of the same maternal origin. Consequently, there remains a degree of uncertainty in that the results of some of these studies might have been influenced by a different paternal (or even maternal) origin of larvae. This might interfere with the influence of food or density on cannibalism by increasing the variability. Such variability exists to a certain extent in our study as well, in spite of the fact that it was conducted on half-siblings. Ideally one would need to study full siblings where both parents are known and presumably the cohort was born on the same day (as used by Pfennig et al., 1994; Alcobendas et al., 2004). We found significant differences in cannibalism levels

even among different larval cohorts (originating from a single female and born on the same day).

Cannibalism and kin

Cannibalism seems to bear on kin relationship: It was found that cannibalistic larvae (*Hynobius retardatus*) avoid killing kin (that originated from the same pond) when given a choice between kin and non-kin (Wakahara, 1997). *Ambystoma opacum* larvae show various aggressive levels toward their kin (hatched out of the same clutch) in comparison to non-kin larvae (Walls and Roudebush, 1991; Walls and Blaustein, 1995; Hokit et al., 1996). *Ambystoma tigrinum nebulosum* larvae developed a cannibalistic morph considerably earlier when non-kin larvae were present (Pfennig and Collins, 1993). There are no such morphs in *S. s. infraimmaculata* larvae. In spite of this, Sadler and Elgar (1994) suggest that there is strong selection against cannibalizing siblings. In *Ambystoma* larvae tactile cues by larvae were shown to be necessary to elicit cannibalism (Hoffman and Pfennig, 1999).

There appears to be an indication that maternal origin is significant when trying to study effect of food and density conditions on cannibalism (although Crump [1992] doubts that there is a purely heritable basis for cannibalism).

The behavioural differences among different cohorts (of different maternal origin) have not been previously studied. We have demonstrated here a significant difference in cannibalism level between different cohorts. These differences suggest that cannibalistic behaviour is also influenced by inherited-genetic factors.

In our study we removed the cannibalistic larvae as soon as they were identified as such. A cannibalistic larva tends to eat all of the other larvae if (when) given a chance. Unless we did so we would have ended up with one big-fat larva (Degani et al., 1980) and as a result we would not have been able to identify the cannibalistic pattern.

Cannibalism and age

The way larval cannibalism changes with age has not been previously studied. Larvae reach the age when the mesonephros is reorganized into a functional kidney and the complete development of the lungs takes place (see Warburg, 1997; Gealekman and Warburg, 2000). The increase in percentage of cannibalistic larvae at the age of 5–6 weeks could be partly related to these changes, including in larval cohorts that are relatively non-cannibalistic (e.g. larval cohort of female G). There seems to be an internal trigger for cannibalism that turns on at the appropriate age regardless of size differences within the cohorts when larvae need to gain extra energy for their differentiation and metamorphic changes.

Does the larval age influence expression of cannibalism (Hoffman and Pfennig 1999)? The fact that after metamorphosis no cannibalistic salamanders were found, even if the salamander had a cannibalistic record as a larva, supports the hypothesis of an age-dependent cannibalism.

Density

As this study was conducted on a limited number of larvae suitable for this study, and as we were limited by the numbers and size of the cohorts available to us, we could not examine the interaction of the two factors (i.e., food availability and individual density). The effect of density on cannibalistic behaviour is very controversial. In the present work we found no evidence that density had any effect on cannibalism level regardless of the amount of food offered. In Table 5 we summarize the food and density conditions of five different studies. These conditions vary greatly between different experiments (Table 5). Thus, high-density conditions were 215.4 cm² per larva in Collins and Cheek, (1983) which is 6.5 fold higher than the low density (16.5–33 cm² per larva) of Wakahara, (1995). Using the same number of larvae at different densities, Degani, (1993) and Ziemba et al., (2000) observed no effect of density on cannibalism. On the

TABLE 5. Food and density conditions in previous experimental studies on larval cannibalism in urodela amphibians. Sources are: a, Collins and Cheek (1983); b, Wakahara (1995); c, Degani (1993); d, Reques and Tejedo (1996); e, present study.

Species	Density (cm ² per larva)			Food (g per larva)			Food item	Source
	Low	Medium	High	Low	Medium	High		
<i>Ambystoma tigrinum nebulosum</i>	176.7 (1)	9		0.050	0.100	0.190	<i>Artemia</i> nauplii	a
	176.7 (1)		215.4 (7)	0.015		0.075	<i>Artemia</i> nauplii	a
	502.7 (3)			0.045		0.225	<i>Artemia</i> nauplii	a
<i>Hynobius retardatus</i>	16.5-33 (5-10)	13.2-16.5 (20-25)	8.25-11 (30-40)			2-3 <i>Tubifex</i>	<i>Tubifex</i>	b
	13.2-16.5 (20-25)			0.5 pellet or <1 <i>Tubifex</i>	1 pellet or 2-3 <i>Tubifex</i>	3 pellet or 4-5 <i>Tubifex</i>	Pellet for frogs or <i>Tubifex</i>	b
<i>Salamandra salamandra</i>	75 (20)	43.75 (20)	7.5 (20)			ad-libitum	Aquatic invertebrates	c
	300 (2)		150 (4)			ad-libitum	Pond zooplanktons	d
	28.65 (4-5)		14.3-15.9 (9-10)	0.005		0.020	Chopped beef liver	e
	28.65 (4-5)		14.3-15.9 (9-10)	0.004	0.010	ad-libitum	<i>Tubifex</i>	e

other hand, Wakahara, (1995), Michimae and Wakahara (2001) and Wildy et al., (2001) found that cannibalism increases with density by using different number of larvae to create different densities.

It seems to us that the nature of the surrounding larval population (number, kinship) rather than sheer densities may affect cannibalism. Thus, not only food and/or density affect cannibalistic behaviour, but the kinship environment affects the onset of cannibalism (Pfennig and Collins, 1993). Since larvae relate to each others' presence and not solely to density, we cannot exclude the possibility that in some (perhaps most) of these studies (including Degani et al., 1980) inter-rather than intra-cohort cannibalism was studied as discussed above.

Food

Likewise, food conditions vary between the different studies with respect to both the food source and its amount (Table 5). When only one *A. t. nebulosum* larva was in the container, it did not develop into a cannibalistic morph regardless of the amount of food offered (Collins and Cheek, 1983). Moreover, they found no effect of food even under other density conditions; therefore, they concluded that food had no effect on cannibalistic behaviour. In other studies (Wakahara, 1995; present study), food had some influence on cannibalistic behaviour. Whereas Wakahara (1995) and Michimae and Wakahara (2001) found that cannibalism increases when food is scarce and decreases when food is abundant, in the present study no such relationship was found. In *S. s. infraimmaculata*, the cannibalism level was similar both when food was scarce and when fed ad-libitum ($P > 0.07$), whereas larvae offered a high food level were significantly more cannibalistic. This supports Walls' (1998) findings that food rather than density has a greater effect.

Comparing rates of cannibalism at low and high food levels, we can conclude that cannibalism increases when food is abundant, whereas by comparing rate of cannibalism

under high and ad-libitum food conditions, we can reach the opposite conclusion. This may be one of the reasons for the different conclusions regarding the effects of both food and density on cannibalism that were reached in all these different studies. We cannot exclude the possibility that there is no 'one rule' of behaviour for all species and that the response for certain environmental conditions varies.

Even within the same species, different conclusions on cannibalistic behaviour could result from experiments conducted with non-cohorts (originating from different mothers) or when sibling larvae of different ages are studied.

Environmental factors such as food and density may both have some effect on cannibalism among salamander larvae. In the present study we have shown for the first time the differences in cannibalism levels among different cohorts thereby implying that some of the major factors are possibly of maternal origin (inherited?). Every cohort has a different cannibalism level; some are more aggressive than others. Moreover, cannibalistic behaviour was found to change with larval age, peaking at the age of 5–6 weeks and then decreasing.

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