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INTRASPECIFIC AGGRESSIVE BEHAVIOUR IN FIRE SALAMANDER LARVAE (*SALAMANDRA SALAMANDRA*): THE EFFECTS OF DENSITY AND BODY SIZE

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The most striking social behaviour in urodele tadpoles is overt aggression against conspecific or heterospecific larvae that may result in harmful injuries, predation or cannibalism. In this study we analysed under laboratory conditions aggressive behaviour in fire salamander, *Salamandra salamandra*, larvae. The aims of our study were: (1) to analyse the function of visual and movement displays in the context of agonistic behaviour; (2) to examine whether relative body size between individuals influences the frequency of aggressive interactions; and, (3) to determine the effect of larval density on the frequency of these aggressive interactions. The analysis of patterns that preceded a direct act of aggression (i.e. lunge or bite) revealed that the initiator of the contest exhibited more attacking patterns and that the receiver displayed more escape acts. Relative body size significantly affected the frequency of aggressive acts. The proportion of aggressive acts performed by the larger larva was positively related to relative larval body size. Density significantly affected the number of aggressive interactions observed. Significantly more aggressive acts were received or displayed by the focal larva at high density. Body size seems to be an important cue which is correlated with the outcome of aggressive interactions in *Salamandra salamandra* larvae. By visually assessing size asymmetry, individuals are able to use this difference to assess fighting ability, and can adjust their behaviour accordingly to avoid escalation of the aggressive encounter.

INTRODUCTION

Social behaviour has been widely studied in anuran tadpoles, especially schooling and sibling recognition (Blaustein *et al.*, 1987; Blaustein, 1988; Waldman, 1991). However, this is not the case for tailed amphibians and only recently have some studies dealt with social behaviour of larval caudates (Walls and Roudebush, 1991; Walls & Semlitsch, 1991; Pfennig & Collins, 1993). The most striking social behaviour in caudate larvae is overt aggression against conspecific or heterospecific larvae. These behaviours are regarded as mechanisms of interference competition that may regulate populations of larval salamanders (Smith, 1990; Van Buskirk & Smith, 1991). Sometimes, the outcome of overt aggression may lead to harmful injuries (Walls & Jaeger, 1987; Semlitsch & Reichling, 1989), or even predation (Wilbur, 1972; Stenhouse *et al.*, 1983; Stenhouse, 1985), or cannibalism (Degani *et al.*, 1980; Kusano *et al.*, 1985; Harris, 1987). The inherent cost of aggression is a potential decrease in survival and growth rates, which would favour the evolution of ritualized behaviour patterns and ameliorate these costs. The relative size of contestants is important in the decision to escalate encounters to overt and aggressive behaviour, and game theory predicts an increase in escalation when relative fighting abilities do not differ between opponents (Parker, 1974). Population density may affect the degree of aggressive interactions, i.e.

injury rates increase in high-density experimental and natural populations of larval salamanders (Semlitsch & Reichling, 1989; van Buskirk & Smith, 1991).

In this study, we experimentally analysed aggressive behaviour of larval fire salamanders, *Salamandra salamandra*, under laboratory conditions. This species is a widespread salamandrid occurring from Western Europe to the Middle-East and North Africa (Thiesmeier, 1992). In the southernmost part of its distribution, the fire salamander breeds in temporary ponds that typically fill after autumn or winter rains and become dry at the end of spring (Degani & Warburg, 1978; Bas-López, 1982; Díaz-Paniagua, 1986; Warburg, 1986*a,b*, 1992). Breeding activity is not continuous but is limited to some peaks, which depend mainly on rainfall (Warburg, 1992; personal observations). This pulse pattern produces variation in larval density across the season and discrete age cohorts that differ in size. Moreover, differential growth rates among tadpoles may increase the range in body size. Aggressive interactions during the larval stage may be frequent, based on the high frequency of injuries observed in the ponds, although other predators may induce it (personal observations). Moreover, cannibalism has been reported in larval fire salamanders (Degani *et al.*, 1980; Warburg, 1992; personal observations) and therefore, aggression may be important in the regulation and dynamics of salamander populations. The objectives of our study were to: (1) analyse the function of visual and movement displays

in the context of agonistic behaviour; (2) examine whether relative body size between individuals influences the frequency of aggressive interactions; and (3) analyse the effect of larval density on the frequency of these aggressive interactions.

MATERIAL AND METHODS

EXPERIMENTAL DESIGN

During early January 1992, we captured salamander larvae from a pond located in Sierra Morena (Córdoba province, Spain). Larvae were of approximately similar age, about one month old. Births were synchronized at this site, coinciding with rains which filled the pond during the first week of December. After that peak, low night temperature prevented female migrations to the pond. Average larval snout-vent-length (SVL) was 22.6 ± 3.3 mm (mean \pm 1 SD; $n = 114$). All larvae were kept in the laboratory in two aquaria of 90 l that were aerated and maintained at room temperature (water temperature $10.5 \pm 2.1^\circ\text{C}$), and maintained under a natural photoperiod. Salamanders were kept in large glass aquaria (50 l) feeding them with pond zooplankton *ad libitum* prior to and during the experiments.

We designed two sets of experiments. Firstly, we addressed whether relative body size affected the frequency of aggressive interactions and whether larger animals performed most of the aggressive acts. We conducted 21 trials, and for each trial two larvae were randomly selected from the storage tanks and introduced in 20 x 15 cm glass aquaria filled with 3 l of pond water. We did not provide any vegetation or hiding places. After 15 min of acclimation, we recorded the sequence and frequency of behavioural patterns for each individual, using spot patterns to distinguish the larvae. No larva was tested more than once. In a second experiment, we asked whether the frequency of aggression events was influenced by larval density. We used two density levels: two or four larvae were introduced into a 30 x 20 cm aquaria filled with 6 l of pond water. For each trial, we randomly selected the larvae from the storage aquaria. After acclimation for 15 min, we randomly selected an individual salamander and recorded all aggressive acts (Bite and Lunge) that it received or performed. Each trial lasted 30 min. Each density was replicated 12 times, and no larva was tested more than once.

QUANTIFICATION OF BEHAVIOUR PATTERNS

We found six different behaviour patterns in larval *S. salamandra* that were similar to those reported by Walls & Jaeger (1987) in ambystomatid larvae. We operationally defined the following: (1) Move toward (MT), one salamander moves by walking in the direction of another; (2) Look toward (LT), one salamander turns its head in the direction of another salamander; (3) Move away (MA), as a consequence of move to-

ward by a second larva, a salamander avoids the contact and increases the distance from it; (4) Look away (LA), a salamander turns its head away from an approaching larva. Two forms of overt aggression were found: (5) Bite (B), one salamander grasps another on the tail, mouth, legs, etc.; and (6) Lunge (L), one salamander moves by fast swimming, not walking, to a second salamander and moved its mouth as in grasping. Bite is an obvious act of aggression (Jaeger, 1984), and Lunge may be considered an unsuccessful bite because the salamander opens and closes its mouth when approaching its opponent (Walls & Semlitsch, 1991). The function of other patterns were categorized as aggressive behaviour (MT and LT) or submissive behaviour (MA) in larval *Ambystoma* (Walls & Semlitsch, 1991). To ascertain whether *Salamandra* has similar patterns of behaviour to that reported for *Ambystoma*, we analysed the acts previous to and subsequent to an act of overt aggression (L or B). We hypothesized that the individual producing the overt aggression would exhibit other aggressive behaviour both before and after the act, and the recipient would show submissive behaviour (Jaeger, 1984; Walls & Semlitsch, 1991). The behaviour of both aggressor and recipient were compared using two-tailed Binomial tests (Siegel, 1956). To avoid pseudoreplication we only analysed the first aggressive act for each trial. The effect of relative body size (SVL of larger salamander/SVL of smaller) on the frequency of aggressions (B and L) was analysed by Spearman rank correlations. All trials lasted 30 min after which the SVL of each larva was measured to the nearest 0.5 mm. No larva was tested more than once.

RESULTS

On average, larger individuals within a pair exhibited a greater number of patterns per trial (large larvae mean = 11.5, SD = 4.4 patterns; small larvae, mean = 8.3, SD = 4.9 patterns; Mann-Whitney *U*-test, $U=122$, $Z=2.48$, $P=0.007$). When the mean frequency of patterns per trial were compared between small and large larvae, significantly more Bites and Lunges were performed by larger larvae. Moreover, greater numbers of LT and MT patterns were displayed by larger larvae. In contrast, smaller salamanders showed higher average frequencies of LA and MA patterns, (Mann-Whitney *U*-test, $P < 0.05$, Fig. 1). Relative body size significantly affected the frequency of aggressive acts ($r_s = -0.655$, $P = 0.0015$, $N = 21$). Higher frequencies were found when the larvae were more similar in size, and frequencies of behaviours dropped to a relatively constant value when the size ratio was greater than 1.2 (Fig. 2a). The proportion of aggressive patterns displayed by the larger larva was positively related to relative larval body size ($r_s = 0.655$, $P = 0.0011$, $N = 21$, Fig. 2b). Larger individuals monopolized aggressive interactions when larvae differed in size. With the exception of three trials, all aggression was performed

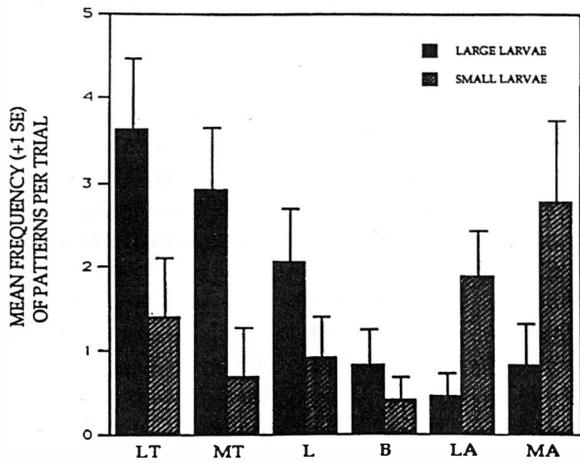


FIG. 1. The mean frequency of patterns in 30 minutes trials ($N=21$) (+ 1 SE) between large and small larvae of *Salamandra salamandra*. LT, look toward; MT, move toward; L, lunge; B, bite; LA, look away; MA, move away.

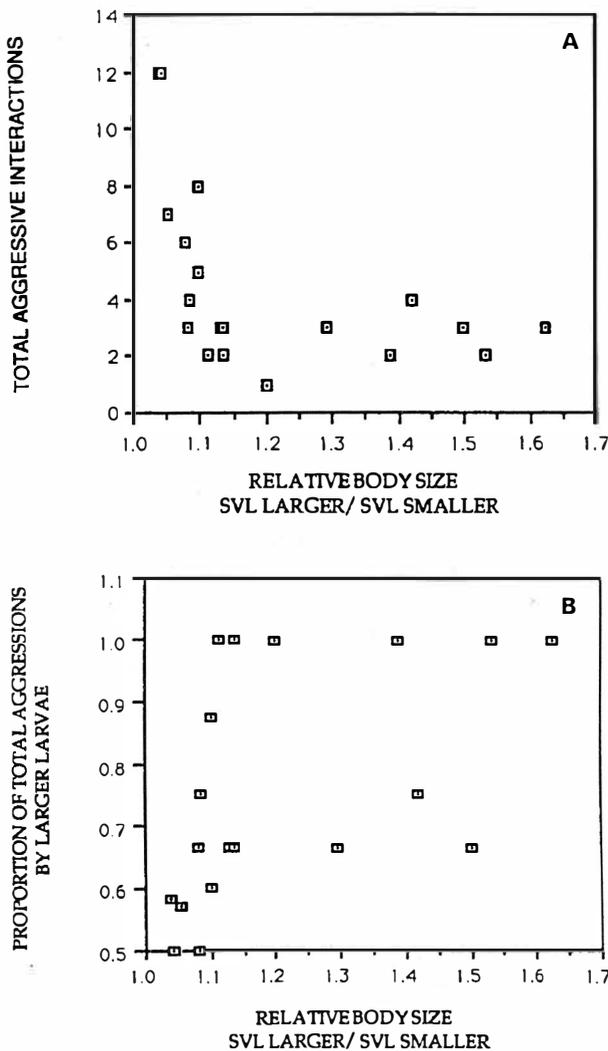


FIG. 2. A, Total number of aggressive acts (Lunge and Bites) per trial as a function of relative body size of larval salamanders (SVL larger/ SVL smaller) ($r_s = -0.655$, $P = 0.0013$, $N = 21$). B, Proportion of total aggressive acts by larger larvae (Lunge and Bites) per trial as a function of relative body size of larval salamanders (SVL larger/ SVL smaller), ($r_s = 0.632$, $P = 0.0021$, $N = 21$).

TABLE 1. Analysis of agonistic patterns before or after to (1) Lunge or (2) Bite. P : probabilities associated with a two-tailed Binomial tests. Missing cells correspond to comparisons with insufficient sample size for assigning probabilities. $N=21$ trials. Ag: aggressor; Re: recipient.

	Before LUNGE			After LUNGE		
	Ag	Re	P	Ag	Re	P
Look Toward	14	3	0.003	6	0	0.032
Move Toward	7	2	0.180	10	1	0.012
Look Away	0	9	0.040	1	2	—
Move Away	0	5	0.062	0	14	<0.001

	Before BITE			After BITE		
	Ag	Re	P	Ag	Re	P
Look Toward	3	2	>0.05	3	1	>0.05
Move Toward	7	1	0.070	3	0	—
Look Away	0	3	—	0	2	—
Move Away	0	2	—	0	7	0.016

by the larger individual above the 1.2 size ratio. This same trend was found when we analysed only frequency of Bite. Bite also exhibits a negative correlation with relative body size ($r_s = -0.476$, $P = 0.029$, $N = 21$), and the proportion of bites given by larger larvae was positively related to relative larval body size ($r_s = 0.70$, $P = 0.016$, $N = 11$). The analysis of the patterns that preceded a Lunge revealed that the aggressor of the contest exhibited significantly more LT than LA and MA acts. Moreover, the recipient displayed more LA and MA acts prior to the Lunge (Table 1a). The analysis of behaviour before and after a Bite was limited because this behaviour occurred infrequently. Before a Bite, only MT differed significantly between aggressors and recipients. Larvae that were bitten subsequently displayed significantly more MA than did the attacker (Table 1b).

Density significantly affected the number of aggressive interactions (Bites and Lunges) observed. Significantly more aggressive behaviours were received or displayed by the focal larva at high density (mean = 9.5, SD = 5.4, $N = 12$) than at low density (mean = 2.5, SD = 1.97, $N = 12$; Mann-Whitney U -test, $Z = 3.138$, $P = 0.0017$, $N = 24$). Therefore, an increase in density promoted higher encounter rates leading to the higher observed rates of aggressive behaviour.

DISCUSSION

Relative body size is an important variable correlated with the outcome of aggressive interactions in larval *Salamandra salamandra*. By visually assessing size asymmetries, individuals are able to use this difference to assess fighting ability, behaving accordingly and not escalating (Maynard Smith & Parker, 1976). The functional aspect of Move Away pattern seems to be a response to aggressive behaviour. After an attack, Lunge or Bite, the recipient usually followed with a Move Away, thus avoiding additional aggression. It is more difficult to ascertain the aggressive role of Move Toward before an attack. Obviously, a larva has to approach another to engage in a Bite or Lunge but, sometimes, the receiver behaved in the same way before it received a Bite or Lunge. In this sense, the function of this pattern may not be clearly dissuasive. This behaviour may be in some sense unavoidable since salamanders react actively to any movement produced in the water and Move Towards pattern may be part of larval predatory behaviour. Larger individuals more frequently exhibited Bite and Lunge than did their smaller opponents, and the relative body size of contestants affected both the total number and proportion of attacks performed by the larger of the pair. Larger larvae were able to monopolize nearly all attacks when the size ratio was greater than 1.2; smaller larvae tended to avoid counter-attacking when the difference in size with its opponent was higher. When size asymmetry decreased the opportunity of being the initiator of an attack did not differ from chance. In ambystomatids, unidirectional aggression was observed when larvae differed largely in size but reciprocated attacks were common when the difference in size between larvae was not significant (Walls & Semlitsch, 1991).

Level of aggression increased significantly with density. The total number of aggressive acts performed or attacks received by focal individuals was greater at the higher density. In natural ponds, situations of crowding may be common and aggression may be frequent. The cost of aggression may be higher due to intrinsic energetic costs associated with it and, moreover, the cost associated with injuries (Harris, 1987). Injuries may result in lower growth rates (Semlitsch & Reichling, 1989), increased susceptibility to predation (Wilbur & Semlitsch, 1990) and/or lower survival due to infections (Walls & Jaeger, 1987). Aggression may function as a mechanism of spacing, but agonistic behaviour against conspecifics may also be a form of predation or cannibalism that has been observed in some salamander populations (Degani *et al.*, 1980; Warburg, 1992; Thiesmeier, 1992; personal observations). In temporary ponds, any advantage derived from larger body size such as superior competitive ability and rapid growth may influence larval fitness traits such as time and size at metamorphosis (Wilbur & Collins, 1973). Some studies have suggested that

larger animals are superior to smaller ones in interference competition (Schoener, 1983; Persson, 1985). Smith (1990) studied the effect of relative body size on the outcome of interference competition in larval salamanders. In these laboratory experiments, Smith found that growth rates were higher for those individuals raised with similar-sized larvae whereas lower growth was found for those larvae raised with larger ones. He could control the level of exploitative competition and interference, through aggressive interactions, was the only mechanism responsible for the depressed growth of smaller larvae. In contrast, our results with *Salamandra* indicate that less aggression was involved as the size difference of larvae increased. If we assume that aggressive behaviour incurs a cost which is a function of the number of interactions, we can expect that the effect of interference competition would be less intense as larvae diverge in size. However, this suggestion does not take into account that the cost of injury may depend on the size-ratio of competing larvae. The fact that smaller larvae did not escalate to injurious attacks when the size of the opponent was relatively high would indicate that the cost induced by injuries is significantly high. Experiments in laboratory or in artificial enclosures are necessary to address the effect of variation in body size has on competition in *Salamandra salamandra* larvae.

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