OIKOS 71: 459–468. Copenhagen 1994

# Does kinship influence density dependence in a larval salamander?

**Susan C. Walls and Andrew R. Blaustein**

Walls, S. C. and Blaustein, A. R. 1994. Does kinship influence density dependence in a larval salamander? – Oikos 71: 459–468.

We examined the effects of variation in larval density, genetic relatedness and their interaction in the marbled salamander, *Ambystoma opacum*. In a factorial experiment, we reared larvae in artificial ponds at low and high densities and in groups comprised of either a single sibship (all siblings) or an equal mixture of six different sibships. After 150 d (before the onset of metamorphosis), we measured the proportion of larvae surviving, body size (snout-vent length and mass), and the size distribution of larvae in each pond. High initial densities significantly reduced body mass, but had no significant effect on larval snout-vent length, the size distributions of individuals, or survival. Neither variation in genetic relatedness (single vs mixed sibship groups), nor its interaction with initial density, influenced larval performance. Because larval survival was independent of initial density, we also assessed whether body size variables were primarily a function of the final number of survivors in each pond. Simple linear regression analyses revealed that larval snout-vent length and mass were negatively related to final density, whereas the skew in the size distributions of individuals increased with increasing final density. Separate regression analyses for groups of siblings vs groups of mixed relatedness revealed no significant differences in the regression slopes and intercepts for these two groups, thus indicating that both estimated the same regression population. Therefore, kinship did not appear to influence density-dependent larval performance in the environment of our artificial ponds. Our results contrast with evidence of kinship effects on behavior under laboratory conditions for this and other species of *Ambystoma* and illustrate the context-dependency with which kin recognition may operate under variable experimental conditions.

*S. C. Walls and A. R. Blaustein, Dept of Zoology, Oregon State Univ., Cordley Hall 3029, Corvallis, OR 97331–2914, USA (present address of SCW: Dept of Biology, The City College of The City Univ. of New York, Convent Avenue at 138th Street, New York, NY 10031, USA).*

A reduction in correlates of fitness (e.g., survival, body size and fecundity), due to enhanced intraspecific competition with increasing density, is a prevailing ecological pattern in many populations. For some organisms, the genetic relatedness of interacting individuals influences the intensity of such density-dependent competition. Based upon Hamilton's (1964a, b) theory of inclusive fitness, individuals may be expected to compete more intensely with unrelated individuals, rather than with siblings (Waldman 1988). Thus, the direction of intraspecific competition toward unrelated or distantly related individuals and away from close relatives could be driven by kin recognition and maintained evolutionarily by kin selection. Alternatively, patterns of resource utilization may be similar among related individuals (e.g., siblings) because of their close genetic relatedness, thus leading to intensified competition among close kin. Evidence from experimental populations lends support to both of these predictions. For example, density-dependent reductions in growth were more severe in groups of mixed geno-

Accepted 28 April 1994


ISSN 0030-1299

types than in pure sibling groups of houseflies (*Musca domestica*) (Bhalla and Sokal 1964) and flour beetles (*Tribolium confusum*) (Jasieński et al. 1988), yet results were mixed for some species of plants (Schmitt and Antonovics 1986, Schmitt and Ehrhardt 1987, Willson et al. 1987).

Larval amphibians have been a model system for examining the nature of density dependence and the effects of genetic relatedness on this phenomenon. Density-dependent growth, survival and metamorphosis have been well documented in the larvae of many anurans (frogs and toads) and salamanders (reviewed in Walls 1991). Thus, there is a strong ecological foundation for examining kinship interactions in the context of competitive growth and development. In anurans, kin-biased behavior occurs in a diversity of species, yet its influence on larval growth and development varies among species and with the context in which it is expressed (Blaustein and Waldman 1992). In two species (*Rana arvalis* and *R. cascadae*), growth is inhibited in sibling groups, compared to that in groups of mixed relatedness (Shvarts and Pyastolova 1970, Hokit and Blaustein, in press). Conversely, growth is enhanced in sibling groups of *Pseudacris triseriata* (D. C. Smith 1990) and, generally, in *Rana sylvatica* (Jasieński 1992). Results for *Bombina variegata* and *Bufo americanus* are variable, with growth either enhanced, inhibited or unaffected when individuals are reared with siblings (Jasieński 1988, 1992, Waldman 1986, 1991).

These studies illustrate the potential importance of kinship to ecological interactions in larval anurans. Kinship is also known to influence behavioral and morphological plasticity in the aggressive and cannibalistic larvae of some salamanders (*Ambystoma opacum*: Walls and Roudebush 1991, Walls and Blaustein unpubl.; *A. tigrinum*: Pfennig and Collins 1993, Pfennig et al. 1994), as well as larval growth in *A. maculatum* (Jasieński 1992). In larval salamanders of the genus *Ambystoma* (Ambystomatidae), density dependence is evident from a number of experiments conducted in both natural ponds and replicated mesocosms (e.g., Wilbur 1972, 1976, Stenhouse et al. 1983, Stenhouse 1985, Petranka and Sih 1986, Semlitsch 1987a, b, Petranka 1989, Scott 1990, C. K. Smith 1990, Van Buskirk and Smith 1991, Sredl and Collins 1992). Yet, the ecological and evolutionary importance of kinship interactions outside the laboratory has yet to be examined, as has been performed with some anuran tadpoles (e.g., Waldman 1982, O'Hara and Blaustein 1985).

We examined the potential effects of the genetic relatedness of cohabiting *Ambystoma opacum* on larval attributes. Larvae of this species exhibit density-dependent growth and survival (Stenhouse et al. 1983, Stenhouse 1985, Petranka 1989, Scott 1990, C. K. Smith 1990) and kin recognition is evidenced by reduced acts of aggression toward siblings (Walls and Roudebush 1991). Thus, we considered *A. opacum* to be an excellent model organism for examining whether kinship affects density-dependent interactions. Such an investigation is essential because differential behavior is not sufficient to conclude a consequence for inclusive fitness which, in turn, is essential to regard interacting individuals as competitors (Peckarsky and Cowan 1991).

To evaluate whether kinship effects, in the context of larval growth and development, may be detected outside of laboratory aquaria, we conducted experimental manipulations of larval density and genetic relatedness in artificial ponds. Such experimental units function as discrete mesocosms that represent "analogs of temporary ponds" (Morin 1983) and have been widely used in studies of amphibian population and community ecology (e.g., see Walls 1991 for a review). However, to our knowledge, they have not been used previously to examine the role of kinship in ecological interactions. We tested the hypothesis that kinship influences density-dependent growth and survival in the spatially complex environment of artificial ponds (compared with laboratory aquaria). Specifically, we predicted that the mean growth and survival of sibling groups would differ from that of genetically mixed groups (i.e., larvae from a combination of different sibships).

# Methods

## Natural history of Ambystoma opacum

The marbled salamander, *Ambystoma opacum* (Ambystomatidae), is broadly distributed throughout much of the eastern United States, ranging from southern New Hampshire to northern Florida, and west to southern Illinois, southeastern Oklahoma, and eastern Texas (Conant and Collins 1991). In autumn, females migrate to the dry basins of temporary woodland ponds, where they each may construct a shallow nest underneath a log or leaf litter and subsequently deposit 16–326 ($\bar{x}$ = 82.1) single eggs (Jackson et al. 1989). Such breeding sites may support numerous groups of siblings from different parents: Jackson et al. (1989) documented a breeding population of more than 8 500 individuals and discovered a total of 95 nests of *A. opacum* during one breeding season in South Carolina, USA. Following oviposition, females guard their eggs until winter rains fill the ponds, when the developing larvae are inundated and hatch. The larval period may last 4–7 mo (Noble and Brady 1933, Petranka 1989, Scott 1990, C. K. Smith 1990). During this time, larvae in some populations are known to interact via interference as intraspecific competitors and predators (Petranka 1989, C. K. Smith 1990), and these behaviors may be mediated by kin discrimination (Walls and Roudebush 1991).

## Collection and pre-experimental maintenance of animals

We collected developing embryos of *Ambystoma opacum* from a natural oviposition site in Oktibbeha Co., Mississippi, USA, on 26 October 1992. Six sibgroups (i.e., clusters of developing eggs, contained in an excavated depression and brooded by a single female) were collected from underneath surface objects covering the dry basin of the temporary pond site (Walls and Altig 1986). Multiple clutches (> 1 female attending a communal group of eggs) were encountered, but were not collected. Each brooding female was replaced in its original position after removal of her clutch. From the time of collection until hatching, each sibgroup was housed individually (see below).

We returned embryos to the laboratory and maintained them, by clutch, on moistened paper in covered dishes (9.2 cm diameter, 4.6 cm deep). Embryos were refrigerated for 4–6 weeks at 4°C until all within a clutch reached the hatching stage (stage 45: Harrison 1969). At this time, we placed larvae within a clutch in dechlorinated water to hatch. Because the dates at which larvae reached the hatching stage varied among clutches, we maintained early hatching larvae in 38-l aquaria filled with 19 l of dechlorinated water until the larvae from all clutches hatched and the experiment began. To insure that early hatching larvae, later to be reared in groups of mixed relatedness (see below), were exposed to both siblings and nonsiblings during this period, each aquarium was divided in half widthwise by a fibreglass mesh screen (mesh size = 2 mm); this procedure is sufficient to familiarize individuals (Walls and Roudebush 1991). We then placed 10–15 larvae from one clutch on one side of an aquarium and 10–15 larvae from another clutch on the opposite side. For larvae to be reared in single sibship groups (see below), both sides of a partitioned aquarium contained larvae from the same clutch. We maintained early hatching larvae in this manner for, at most, two weeks before initiating the experiment. During this period, we fed larvae brine shrimp nauplii (*Artemia salina*) ad libitum, once per day, and the aquaria were cleaned and replenished with fresh water once per week.

## Experimental design and artificial ponds

We assessed the potential effects of variation in density, genetic relatedness and their interaction on survival and body size of larval *A. opacum*. In a 2 × 2 factorial experiment with randomized complete blocks, we experimentally manipulated two levels of initial larval density (12 vs 24 larvae per artificial pond [see below]) and two levels of genetic relatedness: all larvae in a pond were either from only one of six sibships (level 1) or were a mixture of the six different sibships, with equal numbers of larvae from each (level 2). It is not known whether mixing of individuals from different clutches occurs extensively in nature and, if so, how many sibgroups may be involved in this process. However, females may oviposit in communal nests (Noble and Brady 1933, Petranka 1990), thus increasing the likelihood that larvae from different sibgroups intermingle in nature. Our chosen densities were within the range of hatchling densities (1.19–47.4 larvae/$m^2$) observed for this species in natural ponds (Stenhouse 1985, Petranka 1989, Scott 1990, C. K. Smith 1990). We randomly assigned a replicate of each of the resulting four treatment combinations to each of eight spatial blocks of artificial ponds, thus resulting in a total of 32 replicates. For those treatments consisting of a single sibship (relatedness level 1), six of these blocks each represented a different sibgroup; the remaining two blocks represented two randomly selected sibgroups.

We reared larvae in plastic wading pools (1.0 m diameter, 20 cm deep), which have been used previously for experiments with larval *Ambystoma* (Sih et al. 1988, Figiel and Semlitsch 1990, Huang and Sih 1990, 1991). These pools are considerably smaller than the typical natural sites inhabited by larval *A. opacum* (e.g., Petranka 1989), but are close to the average size of those reported for some populations of *A. laterale* (Van Buskirk and Smith 1991). Moreover, these pools possess greater volumes than do laboratory aquaria, which have been used in previous studies of kinship effects in larval *Ambystoma* (Walls and Roudebush 1991, Pfennig and Collins 1993, Pfennig et al. 1994). Artificial ponds were arranged in a rectangular array in a grove of oak trees on the Lewis Brown Horticulture Farm of Oregon State Univ., Benton Co., Oregon, USA. Thus, larvae were exposed to seasonal variation in photoperiod, rainfall and temperature. From 2–4 December 1992 we filled ponds with well water to a depth of 15 cm. On 5–6 December 1992, we added 400 g of dry leaf litter, collected from the oak grove where the ponds were situated, to each experimental unit. On 13 and 15 December 1992, we collected zooplankton (concentrated with a plankton net) and added a 1-l aliquot to each of the 32 ponds. Pools were not covered, thus permitting colonization of aquatic insects as additional prey items for larval salamanders (e.g., Figiel and Semlitsch 1990, Semlitsch and Gibbons 1990, Jackson and Semlitsch 1993).

We began the experiment by adding larvae, according to their designated treatment, to ponds on 19–20 December 1992, when the last of the larvae hatched. Because some larvae had hatched up to two weeks before the beginning of the experiment, we only assigned larvae hatched at similar times to replicates within a given block, thus incorporating the timing of hatching into any potential block effect. Thus, our blocking regime accounted for potential variation due to hatching date, sibship source (see above), and any spatial differences in environmental variables.

We monitored ponds at least once per week. On each visit, we visually assessed larval development and, before the onset of metamorphosis, we terminated the experiment from 15–18 May 1993 (after 150 d). This endpoint

was within the range of larval periods (116–172 d) for *A. opacum* documented in a three-yr study in South Carolina, USA (Scott 1990). All larvae were dipnetted from the ponds and returned to the laboratory, where we determined the proportion surviving, measured them for snout-vent length (to the nearest 0.5 mm) and weighed them (to the nearest 0.001 g). Individuals were allowed to metamorphose in the laboratory and then returned to their natal pond in Mississippi.

### Response variables and statistical analyses

We assessed any overall effect of our blocking regime, initial larval density, relatedness and a density × relatedness interaction on four response variables using multivariate analysis of variance (MANOVA). The MANOVA is a conservative test of a treatment effect and accounts for correlations among multiple response variables. We tested for a block effect by including the factor BLOCK as the last term in a Type III MANOVA model (SAS Institute 1988, Scott 1990).

The four variables analyzed were: proportion surviving, snout-vent length (SVL), mass and the ratio (in mass) of the largest individual in a pond to the mean of all larvae in the pond. We analyzed this latter variable to determine the size distribution of larvae in each pool; i.e., to evaluate the tendency, particularly in high density populations, for one or a few individuals to grow much larger than the remaining larvae (McLachlan 1983, 1989, Van Buskirk and Smith 1991). Such a skewed size distribution may facilitate acts of cannibalism and other forms of aggressive interference, which may be mediated by the genetic relatedness of cohabiting larvae (Walls and Roudebush 1991, Pfennig and Collins 1993, Pfennig et al. 1994). We calculated this ratio based on mass because previous studies (e.g., Walls et al. 1993) have shown mass to be a more sensitive measure of body size in larval *Ambystoma.* For body size data (SVL and mass), we analyzed replicate means because individuals within a replicate cannot be treated as statistically independent observations (Hurlbert 1984, Wilbur 1987).

Before conducting the MANOVA, we visually inspected data for departures from normality by constructing normal probability plots of both the raw data and of their transformations (angular [arcsine square root] transformation for the proportion surviving and size ratio; logarithmic for SVL and mass: Tabachnick and Fidell 1989). Transformations did not visibly improve the approximation of any of the response variables to a normal distribution; thus, the analyses we present here are of the untransformed variables.

If the MANOVA indicated a significant overall effect of initial density, relatedness or their interaction, we then performed univariate, Type III SS ANOVAs on each of the four response variables to evaluate their potential contributions to this effect. Univariate ANOVAs may serve as an interpretative tool of the results from MANOVA; i.e., they aid in the assessment of which variable (s) may have contributed to a significant multivariate response (Tabachnick and Fidell 1989). However, our interpretation of statistical significance relies upon the MANOVA.

To examine the possibility that larval body size (SVL and mass) and size distributions in ponds (largest/mean ratio) were more a consequence of final, rather than initial, larval density, we also performed simple linear regression analysis of SVL, mass and largest/mean ratio (dependent variables) against the final number of larvae per pond (independent variable). For each of the three dependent variables, the data for single and mixed sibship replicates were first analyzed separately. If there were no significant differences in either the regression slopes or intercepts of these two groups, they were combined to calculate a common regression equation for each of the three dependent variables (Zar 1984). All statistical analyses were performed using the General Linear Models (GLM) procedure of the Statistical Analysis System (SAS Institute 1988).

## Results

After 150 d, larval survival in the artificial ponds ranged from 0–83% of the initial number of larvae placed in each. Of the 32 replicates, four had 0% survival, and only one individual survived in each of three others. This mortality most likely was a consequence of freezing temperatures which occurred one month after we began the experiment and which lasted for about one week. Freezing of ponds inhabited by larval *A. opacum* occurs naturally in more northern regions of this species' range (Worthington 1969, Hassinger et al. 1970) and is known to be a source of larval mortality (Walters 1975, Petranka 1989).

The loss of some experimental ponds was approximately evenly distributed among the four treatments but produced unequal cell sizes for the response variables SVL, mass and the largest/mean ratio. Use of the MANOVA statement in the General Linear Models procedure of SAS requires a balanced design (SAS Institute 1988: 565), which we achieved by randomly eliminating three blocks of replicates (Tabachnick and Fidell 1989). Thus, our balanced design consisted of five replicates of each of the four treatments ($n$ = 20 ponds). For comparative purposes, in terms of significance of the main effects and their interaction, the ANOVA results from this balanced design (see below) were similar to those for an unbalanced design (SAS Institute 1988: 555–557). However, the latter resulted in some block effects and did not allow for simultaneous inference of multiple response variables, as was achieved by the MANOVA with a balanced design.

MANOVA indicated that there was a significant effect of initial density, but not of relatedness nor of an interac-

Table 1. Summary of multivariate analysis of variance (MANOVA) for the effects of block, density, genetic relatedness and a density × relatedness interaction on four response variables in larval *A. opacum*.

| Source of variation | Wilks' Lambda | *F* | df | *P* |
|---|---|---|---|---|
| Block | 0.125 | 1.72 | 16,28 | 0.102 |
| Density | 0.306 | 5.11 | 4,9 | 0.020* |
| Relatedness | 0.617 | 1.40 | 4,9 | 0.309 |
| Density × Relatedness | 0.669 | 1.11 | 4,9 | 0.408 |

* Significant at $\alpha = 0.05$.

tion between these main effects, on the combined response variables (Table 1). There also was no significant overall effect of our blocking regime (Table 1). Thus, the block term (sum of squares and df) was pooled with the residual error term for remaining statistical tests (Cochran and Cox 1957). Subsequent univariate ANOVAs on each of the four response variables indicated that this effect of initial density was due to a significant reduction in larval mass at the higher density (Table 2C, Fig. 1C). Neither the proportion surviving (Table 2A, Fig. 1A), snout-vent length (Table 2B, Fig. 1B), nor the ratio in mass of the largest individual to the mean of each pool (Table 2D, Fig. 1D) was significantly affected by our experimental manipulation of larval density. In interpreting Fig. 1A, it is important to note that the mean proportion surviving is based on $n = 5$, for consistency with the MANOVA/ANOVA results. Mean survival, calculated for all initial eight replicates, was more uniform ($\bar{x}$ + 1 SD single sibship groups, low density vs high = 0.21 + 0.148 vs 0.235 + 0.120; mixed sibship groups, low density vs high = 0.33 + 0.235 vs 0.265 + 0.291). Overall survival for the 32 ponds averaged 26% and was clearly independent of initial density.

Because of this density-independent survival, we questioned whether the *final* number of survivors per replicate, rather than initial density, might more accurately predict larval body size and the size distribution in each

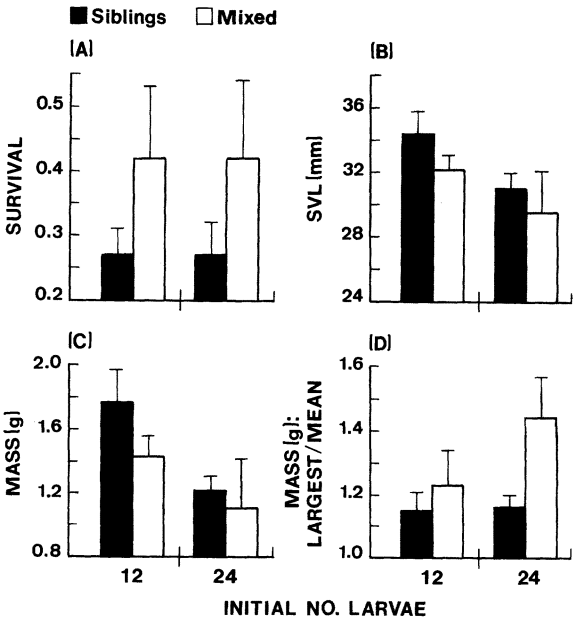


Fig. 1. Mean (+ 1 SE) responses of larval *A. opacum* reared in groups composed of single sibships (solid bars) vs groups of mixed sibships (open bars), at low (12 individuals per pool) and high (24 individuals per pool) initial densities. Means shown are for a reduced sample size of five replicates for each of the four experimental treatments (total $n = 20$), which corresponds to the MANOVA/ANOVA results for a balanced design depicted in Tables 1 and 2. The response variables illustrated are (A) the proportion of larvae that survived to the end of the experiment, (B) snout-vent length (SVL), (C) mass and (D) the ratio in mass of the largest individual in a replicate pool to the pool's average.

pond. This relationship seemed particularly plausible, assuming that mortality was associated with the freezing conditions that occurred within the first month of the experiment. To examine this possibility, for each variable we performed simple linear regression analysis on the mean response of each pond for which a mean could be calculated (i.e., those with ≥ 2 surviving larvae; $n$ = 13

Table 2. Summary of univariate analysis of variance (ANOVA) for the effects of density, relatedness, and their interaction on four response variables in larval *A. opacum*.

| Response variable | Source of variation | df | Type III SS | *F* | *P* |
|---|---|---|---|---|---|
| A. Proportion surviving | Density | 1 | 0.000 | 0.00 | 1.00 |
| | Relatedness | 1 | 0.110 | 2.73 | 0.118 |
| | Density × Relatedness | 1 | 0.000 | 0.00 | 1.00 |
| B. Snout-vent length (mm) | Density | 1 | 47.09 | 3.51 | 0.079 |
| | Relatedness | 1 | 17.69 | 1.32 | 0.268 |
| | Density × Relatedness | 1 | 0.853 | 0.06 | 0.804 |
| C. Mass (g) | Density | 1 | 0.916 | 4.82 | 0.043* |
| | Relatedness | 1 | 0.251 | 1.32 | 0.267 |
| | Density × Relatedness | 1 | 0.072 | 0.38 | 0.547 |
| D. Mass (g): Largest/mean ratio | Density | 1 | 0.062 | 1.00 | 0.333 |
| | Relatedness | 1 | 0.157 | 2.53 | 0.131 |
| | Density × Relatedness | 1 | 0.045 | 0.73 | 0.406 |

*Significant at $\alpha = 0.05$.

Table 3. Results of simple linear regression analyses on the final number of larval *A. opacum* per replicate (independent variable) vs snout-vent length (SVL: mm), mass (g), and the ratio (in mass) of the largest individual in a replicate pool to the pool's average (dependent variables). $r^2$ = coefficient of determination; $F$ = test statistic for analysis of variance of regression model, performed separately for sibling and mixed groups; $t$ = statistic from t-test comparison of sibling and mixed groups for homogeneity of their slopes and intercepts; $P$ = level of significance, two-tailed. $n$ = 13 and 12 for groups of siblings vs groups of mixed relatedness, respectively. "–" indicates that tests between groups for homogeneity of their regression parameters were not performed because of a nonsignificant regression within each group.

| Statistic/Parameter | Dependent variable | | | | | |
|---|---|---|---|---|---|---|
| | SVL | | Mass | | Ratio | |
| | Sibs | Mixed | Sibs | Mixed | Sibs | Mixed |
| $r^2$ | 0.38 | 0.59 | 0.48 | 0.67 | 0.22 | 0.18 |
| $F$ | 6.83 | 14.63 | 10.12 | 20.47 | 3.16 | 2.20 |
| $P$ | 0.0241 | 0.0033 | 0.0087 | 0.0011 | 0.1030 | 0.1691 |
| Intercept | 36.70 | 35.34 | 2.11 | 1.84 | 1.06 | 1.13 |
| $t$ | 0.28 | | 0.05 | | – | |
| $P$ | > 0.05 | | > 0.05 | | – | |
| Slope | –0.81 | –0.60 | –0.12 | –0.07 | 0.02 | 0.03 |
| $t$ | –0.57 | | –1.21 | | – | |
| $p$ | >0.05 | | >0.05 | | – | |

pure sibling groups and 12 mixed sibship groups). Snout-vent length and mass were significantly negatively related to final larval density for both single and mixed sibship groups (Table 3). However, when these two groups were analyzed separately, the size ratio of the largest larva to the mean was not significantly related to final density (Table 3). For SVL and mass, subsequent t-test comparisons (Zar 1984) between single and mixed sibship groups revealed that neither their regression coefficients (slopes) nor intercepts were significantly different (Table 3). Thus, the genetic relatedness of cohabiting larvae had no discernible influence on final larval size: both single and mixed sibship groups exhibited statistically equivalent patterns of density-dependent growth. We thus considered groups of siblings and groups of mixed relatedness to estimate the same population regression and pooled them to compute a common regression equation (Zar 1984).

Both initial and final densities significantly influenced mean larval mass per replicate, which decreased with increases in each (Fig. 1C and 2B). Although our initial density manipulation had no significant effect on either SVL or the largest/mean size ratio, both of these variables were related to final density. Mean SVL per replicate significantly decreased with increasing final density (Fig. 2A), whereas the largest/mean size ratio per pond was significantly positively related to increasing final density (Fig. 2C). Thus, high final densities resulted in an overall reduction in mean larval body size per pond, but also increased the size of one or a few larvae relative to the average larval size for their group.

Because an increase in final density represents an increase in sample size, it is possible that the occurrence of one or a few unusually large individuals at higher densities is a statistical artifact (i.e., with increasing sample size, there is a concomitant increase in the likelihood that a given sample will contain one of these individuals. Based upon this argument, we predicted that the size of the largest individual in a replicate should significantly increase with increasing final density (i.e., sample size). Contrary to this prediction, a simple linear regression analysis revealed that the mass of the largest individual in each replicate significantly *decreased* with increasing density ($r$ = –0.70; $F$ = 21.659; $P$ = 0.00011). Thus, it seems unlikely that the inclusion of large individuals was due to random chance in sampling a larger number of individuals.

## Discussion

Under the conditions of our experiment, we failed to detect any effect of kinship on the growth and survival of larval *A. opacum.* Survival was density-independent, most likely because of pond freezing that occurred early in the larval period. Despite this mortality, final densities were sufficiently high in some replicates for larvae to exhibit negative density-dependent growth. Moreover, increasing final density significantly increased the size of a few larvae relative to the mean body size of a group. Such skewed size distributions at higher densities have been observed in both natural and experimental larval populations of *Ambystoma laterale* (Van Buskirk and Smith 1991), as well as in some aquatic insects (McLachlan 1983, 1989, Peckarsky and Cowan 1991).

In larval salamanders, aggressive superiority and the potential for cannibalism increases as size disparities among individuals increase (C. K. Smith 1990, Walls and Semlitsch 1991). Size differences between pairs of larval *A. maculatum* were significantly greater for unrelated larvae than for siblings, although the incidence of injury did not differ between these two groups (Jasieński 1992). Additionally, the negative influence of larger *A. mac-*

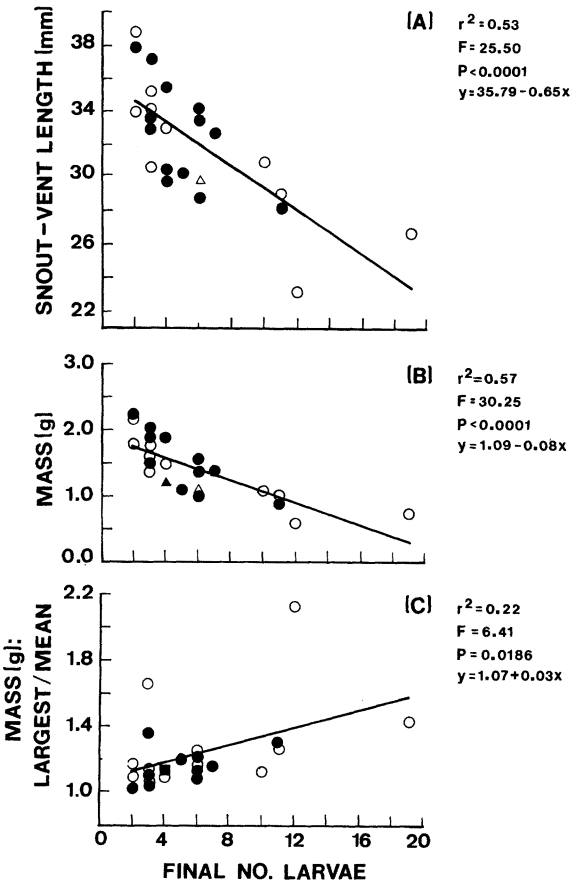


Fig. 2. Results of simple linear regression analyses of the number of larval *A. opacum* remaining at the end of the experiment (independent variable) vs (A) snout-vent length, (B) mass and (C) the ratio in mass of the largest individual in a replicate pool to the pool's average (dependent variables). Each point represents the mean response for a single replicate experimental pool. Only those replicates for which a mean value could be calculated (i.e., those with at least two survivors; $n = 25$) are depicted. Solid symbols represent responses for pools containing individuals from a single sibship ($n = 13$); open symbols represent responses for pools containing individuals from a mixture of sibships ($n = 12$). Symbols indicate one (●, ○), two (▲, △), or three (■, □) overlapping values. Regression results are for the combined responses of single vs mixed sibship groups, because these two samples estimated the same population regression (Table 3).

*ulatum* on the growth of smaller conspecifics was significantly more pronounced in unrelated pairs (Jasieński 1992). Moreover, in a laboratory study of larval *A. tigrinum*, the development of a cannibalistic morphology was reduced in the presence of siblings (Pfennig and Collins 1993). Thus, in our experiment, one might expect kin recognition to have mediated the production of large, potentially cannibalistic (Blaustein and O'Hara 1982) larvae at higher densities, if the genetic relatedness of cohabiting larvae were an important factor in the environment of our artificial ponds. Similarly, Radwan (1993) found that kinship did not influence density-dependent morphogenesis in an acarid mite, in which the production of alternative "fighter" and "non-fighter" male morphs did not differ between groups of siblings versus mixed groups of unrelated individuals. Thus, the influence of kinship on size dimorphism and other types of morphological plasticity remains unclear.

Using a variety of laboratory assays, kin recognition has been demonstrated in three of the four species of *Ambystoma* that have been tested (*A. gracile*: S. Walls and A. Blaustein, unpubl.; *A. maculatum*: Jasieński 1992; *A. opacum*: Walls and Roudebush 1991; *A. tigrinum*: Pfennig and Collins 1993, Pfennig et al. 1994; see review in Blaustein and Walls in press). The reason we did not detect a kinship effect in the present experiment is unclear. The expression of kin recognition is often a consequence of the ecological and social contexts in which it may occur (Blaustein et al. 1987, Beecher 1988, 1991, Reeve 1989, Waldman 1991). Such context-dependency may explain our divergent results, for a number of factors differed in our study.

First, the population source (Mississippi) of larvae we used was different from those used in a previous demonstration of kin recognition in *A. opacum* from Louisiana (Walls and Roudebush 1991). If there is geographic variation in kin recognition in this species, it is possible that such behavior has not evolved in the population that we examined. Moreover, potential differences between the natural habitat and the environment in which larvae were reared (including atypical exposure to freezing conditions) may have influenced our results. Third, in studies of some larval anurans (e.g., D. C. Smith 1990, Hokit and Blaustein, in press), kinship effects were only observed in relatively small chambers at high (albeit natural) initial densities. In our experiment, kinship may have affected growth if larval survival and/or initial densities had been higher. Our final densities ($\bar{x} \pm 1$ SD = 5.8±4.03 larvae/m$^2$; replicates with < 2 survivors excluded) were less than estimates of natural densities (9.5–10.8 larvae/m$^2$) for larval *A. opacum* late in the larval period (C. K. Smith 1990), but were within the range of final densities reported by Petranka (1989) in his Fig. 1. More importantly, our final densities were sufficiently high to cause density-dependent growth. Last, the leaf-litter layer in each of our artificial ponds created spatial refugia from predators. Compared to the chambers used in previous laboratory studies (Walls and Roudebush 1991, Pfennig and Collins 1993, Pfennig et al. 1994), this increased spatial complexity of our ponds (Harris 1987, Sredl and Collins 1992) may have minimized direct aggressive interactions, thus altering the cost-benefit ratio of kin discrimination (Hamilton 1964a, b) in heterogeneous environments.

Density-dependent effects on larval performance, such as those demonstrated in our experiment, may be widespread in *Ambystoma*. However, in many cases, the mechanisms of density-dependence in nature are not known. Chemical interference (i.e., the production of growth inhibitors) has failed to be detected in larval

salamanders (Bell 1974, Petranka 1984, Walls and Jaeger 1989). Alternatively, several authors (Morin et al. 1983, Petranka 1984, 1989, Petranka and Sih 1986, Scott 1990) have documented food (microcrustacean) limitation in both artificial and natural habitats. Others have suggested that larvae are, at most, perhaps occasionally food-limited (Taylor et al. 1988). In natural and experimental populations of larval *A. talpoideum* and *A. laterale*, an increased frequency of larvae with injured tails at higher densities has been convincingly attributed to aggressive attacks (interference) by conspecifics, because of the absence of other potential predators (Semlitsch and Reichling 1989, Van Buskirk and Smith 1991). In *A. opacum*, kinship-mediated aggression occurs (Walls and Roudebush 1991) and density-dependent tail injuries have been reported under natural conditions (Petranka 1989, Scott 1990), although the potential contribution of attacks from insect predators were not quantified in these two studies. In our study, unambiguous indications of injury (i.e., missing tips of tails or limbs) were only evident in 5% of the surviving larvae, though this low incidence does not eliminate the possible role that interference may have played in mortality or earlier injuries.

To advance knowledge of whether kin-biased behavior influences density-dependent interactions in nature, a priority is to elucidate the precise mechanism(s) by which density-dependence is achieved. An important step in this process is to evaluate directly the effects of varying densities on resource exploitation and aggressive interference (e.g., McPeek and Crowley 1987), which has been largely ignored in studies with larval salamanders. Second, we emphasize a need for an alliance between laboratory studies of kinship behavior and experimental studies conducted in nature (e.g., Waldman 1982, O'Hara and Blaustein 1985). Although laboratory studies may reveal the evolutionary potential for such behavior to occur, field experiments demonstrate the potential significance of behavioral phenomena in the natural environment. Such an integrated approach will be essential in formulating a realistic model of how kin recognition influences the inclusive fitness of discriminating larval salamanders in their natural pond habitat.

*Acknowledgements* – We thank R. Altig for access to his study site, and T. Holder for returning metamorphosed salamanders to this site at the completion of our study. We are especially grateful to S. Wise and D. G. Hokit for help in maintaining the experimental pools during a period of freezing temperatures; to S. Rothstein and D. Mock for their comments on an earlier draft of this manuscript; and to R. Rockwell and S. Ratner for advice on statistical analyses. We additionally thank D. Pfennig for access to unpublished manuscripts, and R. Vorgas, S. Vorgas and H. Quinlan for all their help. This research was supported by a National Science Foundation grant BNS-9107172 to ARB and SCW. Additional support was provided by a USDA Forest Service grant to J. Beatty and A. Blaustein.

## References

Beecher, M. D. 1988. Kin recognition in birds. – Behav. Genet. 18: 465–482.
– 1991. Successes and failures of parent-offspring recognition in animals. – In: Hepper, P. G. (ed.), Kin recognition. Cambridge Univ. Press, Cambridge, pp. 94–124.
Bell, G. 1974. The reduction of morphological variation in natural populations of smooth newt larvae. – J. Anim. Ecol. 43:115–128.
Bhalla, S. C. and Sokal, R. R. 1964. Competition among genotypes in the housefly at varying densities and proportions (the *green* strain). – Evolution 18: 312–330.
Blaustein, A. R. and O'Hara, R. K. 1982. Kin recognition in *Rana cascadae* tadpoles: maternal and paternal effects. – Anim. Behav. 30: 1151–1157.
– and Waldman, B. 1992. Kin recognition in anuran amphibians. – Anim. Behav. 44: 207–221.
– and Walls, S. C. In press. Aggregation and kin recognition behaviour. – In: Heatwole, H. and Sullivan, B. K. (eds), Amphibian biology, vol. 3. Surrey Beatty and Sons, Chipping Norton.
– , Bekoff, M. and Daniels, T. J. 1987. Kin recognition in vertebrates (excluding primates): mechanisms, functions, and future research. – In: Fletcher, D. J. C. and Michener, C. D. (eds), Kin recognition in animals. Wiley, New York, pp. 333–357.
Cochran, W. G. and Cox, G. M. 1957. Experimental design. 2nd ed. – Wiley, New York.
Conant, R. and Collins, J. T. 1991. A field guide to reptiles and amphibians: eastern and central North America. 3rd ed. – Houghton Mifflin, Boston.
Figiel, C. R., Jr. and Semlitsch, R. D. 1990. Population variation in survival and metamorphosis of larval salamanders (*Ambystoma maculatum*) in the presence and absence of fish predation. – Copeia 1990: 818–826.
Hamilton, W. D. 1964a. The genetical evolution of social behaviour. I. – J. Theor. Biol. 7: 1–16.
– 1964b. The genetical evolution of social behaviour. II. – J. Theor. Biol. 7: 17–52.
Harris, R. N. 1987. An experimental study of population regulation in the salamander, *Notophthalmus viridescens dorsalis* (Urodela: Salamandridae). – Oecologia 71: 280–285.
Harrison, R. G. 1969. Harrison stages and description of the normal development of the spotted salamander, *Amblystoma punctatum* (Linn.). – In: Harrison, R. G. (ed.), Organization and development of the embryo. Yale Univ. Press, New Haven, CT, pp. 44–66.
Hassinger, D. D., Anderson, J. D. and Dalrymple, G. H. 1970. The early life history and ecology of *Ambystoma tigrinum* and *Ambystoma opacum* in New Jersey. – Am. Midl. Nat. 84: 474–495.
Hokit, D. G. and Blaustein, A. R. The effects of kinship on growth and development in tadpoles of *Rana cascadae*. – Evolution, in press.
Huang, C. and Sih, A. 1990. Experimentally studies on behaviorally mediated, indirect interactions through a shared predator. – Ecology 71: 1515–1522.
– and Sih, A. 1991. Experimental studies on direct and indirect interactions in a three trophic-level stream system. – Oecologia 85:530–536.
Hurlbert, S. H. 1984. Pseudoreplication and the design of ecological field experiments. – Ecol. Monogr. 54: 187–211.
Jackson, M. E. and Semlitsch, R. D. 1993. Paedomorphosis in the salamander *Ambystoma talpoideum*: effects of a fish predator. – Ecology 74: 342–350.
– , Scott, D. E. and Estes, R. A. 1989. Determinants of nest success in the marbled salamander (*Ambystoma opacum*). – Can. J. Zool. 67: 2277–2281.
Jasieński, M. 1988. Kinship ecology of competition: size hierarchies in kin and nonkin laboratory cohorts of tadpoles. – Oecologia 77: 407–413.

– 1992. Kinship effects and plasticity of growth in amphibian larvae. – Unpublished thesis. Harvard Univ., Cambridge, MA.
– , Korzeniak, U. and Łomnicki, A. 1988. Ecology of kin and nonkin larval interactions in *Tribolium* beetles. – Behav. Ecol. Sociobiol. 22: 277–284.
McLachlan, A. 1983. Life-history tactics of rain-pool dwellers. – J. Anim. Ecol. 52: 545–561.
– 1989. Animal populations at extreme densities: size dimorphism by frequency dependent selection in ephemeral habitats. – Funct. Ecol. 3: 633–643.
McPeek, M. A. and Crowley, P. H. 1987. The effects of density and relative size on the aggressive behaviour, movement and feeding of damselfly larvae (Odonata: Coenagrionidae). – Anim. Behav. 35: 1051–1061.
Morin, P. J. 1983. Predation, competition, and the composition of larval anuran guilds. – Ecol. Monogr. 53: 119–138.
– , Wilbur, H. M. and Harris, R. N. 1983. Salamander predation and the structure of experimental communities: responses of *Notophthalmus* and microcrustacea. – Ecology 64: 1430–1436.
Noble, G. K. and Brady, M. K. 1933. Observations on the life history of the marbled salamander, *Ambystoma opacum* Gravenhorst. – Zoologica 11: 89–132.
O'Hara, R. K. and Blaustein, A. R. 1985. *Rana cascadae* tadpoles aggregate with siblings: an experimental field study. – Oecologia 67: 44–51.
Peckarsky, B. L. and Cowan, C. A. 1991. Consequences of larval intraspecific competition to stonefly growth and fecundity. – Oecologia 88: 277–288.
Petranka, J. W. 1984. Sources of interpopulational variation in growth responses of larval salamanders. – Ecology 65: 1857–1865.
– 1989. Density-dependent growth and survival of larval *Ambystoma*: evidence from whole-pond manipulations. – Ecology 70: 1752–1767.
– 1990. Observations on nest site selection, nest desertion, and embryonic survival in marbled salamanders. – J. Herpetol. 24: 229–234.
– and Sih, A. 1986. Environmental instability, competition, and density-dependent growth and survivorship of a stream-dwelling salamander. – Ecology 67: 729–736.
Pfennig, D. W. and Collins, J. P. 1993. Kinship affects morphogenesis in cannibalistic salamanders. – Nature 362: 836–838.
– , Sherman, P. W. and Collins, J. P. 1994. Kinship and cannibalism in polyphenic salamanders. – Behav. Ecol. 5: 225–232.
Radwan, J. 1993. Kin recognition in the acarid mite, *Caloglyphus berlesei*: negative evidence. – Anim. Behav. 45: 200–202.
Reeve, H. K. 1989. The evolution of conspecific acceptance thresholds. – Am. Nat. 133: 407–435.
SAS Institute 1988. SAS/STAT user's guide, release 6.03 ed. – SAS Institute Inc., Cary, NC.
Schmitt, J. and Antonovics, J. 1986. Experimental studies of the evolutionary significance of sexual reproduction. IV. Effect of neighbor relatedness and aphid infestation on seedling performance. – Evolution 40: 830–836.
– and Ehrhardt, D. W. 1987. A test of the sib-competition hypothesis for outcrossing advantage in *Impatiens capensis*. – Evolution 41: 579–590.
Scott, D. E. 1990. Effects of larval density in *Ambystoma opacum*: an experiment in large-scale field enclosures. – Ecology 71: 296–306.
Semlitsch, R. D. 1987a. Paedomorphosis in *Ambystoma talpoideum*: effects of density, food, and pond drying. – Ecology 68: 994–1002.
– 1987b. Density-dependent growth and fecundity in the paedomorphic salamander *Ambystoma talpoideum*. – Ecology 68: 1003–1008.
– and Reichling, S. B. 1989. Density-dependent injury in larval salamanders. – Oecologia 81: 100–103.
– and Gibbons, J. W. 1990. Effects of egg size on success of larval salamanders in complex aquatic environments. – Ecology 71: 1789–1795.
Shvarts, S. S. and Pyastolova, O. A. 1970. Regulators of growth and development of amphibian larvae. I. Specificity of effects. – Soviet J. Ecol. 1: 58–62.
Sih, A., Petranka, J. W. and Kats, L. B. 1988. The dynamics of prey refuge use: a model and tests with sunfish and salamander larvae. – Am. Nat. 132: 463–483.
Smith, C. K. 1990. Effects of variation in body size on intraspecific competition among larval salamanders. – Ecology 71: 1777–1788.
Smith, D. C. 1990. Population structure and competition among kin in the chorus frog (*Pseudacris triseriata*). – Evolution 44: 1529–1541.
Sredl, M. J. and Collins, J. P. 1992. The interaction of predation, competition, and habitat complexity in structuring an amphibian community. – Copeia 1992: 607–614.
Stenhouse, S. L. 1985. Interdemic variation in predation on salamander larvae. – Ecology 66: 1706–1717.
– , Hairston, N. G. and Cobey, A. E. 1983. Predation and competition in *Ambystoma* larvae: field and laboratory experiments. – J. Herpetol. 17: 210–220.
Tabachnick, B. G. and Fidell, L. S. 1989. Using multivariate statistics. 2nd ed. – Harper Collins, New York.
Taylor, B. E., Estes, R. A., Pechmann, J. H. K. and Semlitsch, R. D. 1988. Trophic relations in a temporary pond: larval salamanders and their microinvertebrate prey. – Can. J. Zool. 66: 2191–2198.
Van Buskirk, J. and Smith, D. C. 1991. Density-dependent population regulation in a salamander. – Ecology 72: 1747–1756.
Waldman, B. 1982. Sibling association among schooling toad tadpoles: field evidence and implications. – Anim. Behav. 30: 700–713.
– 1986. Chemical ecology of kin recognition in anuran amphibians. – In: Düvall, D., Muller-Schwarze, D. and Silverstein, R. M. (eds), Chemical signals in vertebrates IV. Ecology, evolution and comparative biology. Plenum Press, New York, pp. 225–242.
– 1988. The ecology of kin recognition. – Annu. Rev. Ecol. Syst. 19: 543–571.
– 1991. Kin recognition in amphibians. – In: Hepper, P. G. (ed.), Kin recognition. Cambridge Univ. Press, Cambridge, pp. 162–219.
Walls, S. C. 1991. Mechanisms of coexistence in a guild of ambystomatid salamanders: the importance of stage-dependent regulation in species with a complex life cycle. – Unpublished thesis. Univ. Southwestern Louisiana, Lafayette, LA.
– and Altig, R. 1986. Female reproductive biology and larval life history of *Ambystoma* salamanders: a comparison of egg size, hatchling size, and larval growth. – Herpetologica 42: 334–345.
– and Jaeger, R. G. 1989. Growth in larval salamanders is not inhibited through chemical interference competition. – Copeia 1989: 1049–1052.
– and Roudebush, R. E. 1991. Reduced aggression toward siblings as evidence of kin recognition in cannibalistic salamanders. – Am. Nat. 138: 1027–1038.
– and Semlitsch, R. D. 1991. Visual and movement displays function as agonistic behavior in larval salamanders. – Copeia 1991: 936–942.
– , Belanger, S. S. and Blaustein, A. R. 1993. Morphological variation in a larval salamander: dietary induction of plasticity in head shape. – Oecologia 96: 162–168.
Walters, B. 1975. Studies of interspecific predation within an amphibian community. – J. Herpetol. 9: 267–279.
Wilbur, H. M. 1972. Competition, predation, and the structure of the *Ambystoma-Rana sylvatica* community. – Ecology 53: 3–21.

– 1976. Density-dependent aspects of metamorphosis in *Ambystoma* and *Rana sylvatica*. – Ecology 57: 1289–1296.
– 1987. Regulation of structure in complex systems: experimental temporary pond communities. – Ecology 68: 1437–1452.
Willson, M. F., Thomas, P. A., Hoppes, W. G., Katusic-Malmborg, P. L., Goldman, D. A. and Bothwell, J. L. 1987. Sibling competition in plants: an experimental study. – Am. Nat. 129: 304–311.
Worthington, R. D. 1969. Additional observations on sympatric species of salamander larvae in a Maryland pond. – Herpetologica 25: 227–229.
Zar, J. H. 1984. Biostatistical analysis. 2nd ed. – Prentice-Hall, Englewood Cliffs, NJ.