

Influence of abundance of small-mammal burrows and conspecifics on the density and distribution of spotted salamanders (*Ambystoma maculatum*) in terrestrial habitats

Jonathan V. Regosin, Bryan S. Windmiller, and J. Michael Reed

Abstract: Spotted salamander (*Ambystoma maculatum*) distributions were studied within eighteen 272-m² field enclosures in upland deciduous forest adjacent to two breeding ponds. The enclosures were subsequently used in experiments involving (i) removal of small-mammal burrows to determine if burrow abundance influenced salamander density and (ii) manipulation of salamander densities to determine if increased density was associated with decreased growth, fecundity, or probability of re-emerging to breed. Mean density of salamanders wintering in enclosures <65 m from the breeding ponds was 1.0/100 m² (SD = 1.1/100 m², n = 17, range = 0–4.0/100 m²). Density declined as distance to the nearest breeding pond increased, and the sex ratio was skewed sharply toward males (8:1) wintering close (<65 m) to the pond but not farther from the pond (1:1). Males residing within the enclosures weighed more than males wintering farther from the breeding ponds. Migrating salamanders were over 3 times more likely to leave burrow-removal plots than control plots prior to the summer inactive period. Salamanders housed at high density (15/enclosure) were less likely to re-emerge to breed than were salamanders housed at low density (5/enclosure), but we observed no effects on growth or fecundity. Results suggest that conspecific distribution and burrow availability might affect the density of spotted salamanders within terrestrial habitats.

Résumé : Nous avons gardé des salamandres tachetées (*Ambystoma maculatum*) en nature, dans des enceintes totalisant dix-huit 272 m² de surface dans une forêt décidue des hautes terres adjacente à deux étangs de reproduction, dans le but d'en étudier la répartition. Les enceintes ont ensuite servi à des expériences au cours desquelles (i) les terriers de petits mammifères ont été enlevés pour évaluer les effets de leur abondance sur la densité des salamandres et (ii) la densité des salamandres a été manipulée pour permettre de déterminer si l'augmentation de la densité est associée une réduction de la croissance et de la fécondité ou de la probabilité d'une nouvelle émergence de reproduction. La densité moyenne des salamandres qui ont passé l'hiver dans des enceintes installées à moins de 65 m des étangs était de 1,0/100 m² (écart type = 1,1/100 m², n = 17, étendue = 0–4,0/100 m²). La densité diminuait en fonction inverse de la distance de l'étang de reproduction le plus proche et le rapport mâles:femelles était beaucoup plus grand que 1 (8 : 1) en hiver près de l'étang (<65 m), mais pas plus loin (1 : 1). Les mâles qui passaient l'hiver dans les enceintes étaient plus lourds que ceux qui résidaient à de plus grandes distances des étangs. Les salamandres migratrices étaient plus enclines à quitter les enceintes d'où les terriers avaient été enlevés que les enceintes témoins avant la période inactive de l'été. Les salamandres tachetées regroupées à des densités élevées (15 individus/enceinte) avaient moins tendance à réémerger pour se reproduire que celles qui étaient regroupées à des densités moins élevées (5 individus/enceinte), mais nous n'avons observé aucun effet de la densité sur la croissance ou la fécondité. Nos résultats semblent indiquer que la répartition de salamandres de la même espèce et la disponibilité des terriers peuvent influencer la densité des salamandres tachetées des milieux terrestres.

[Traduit par la Rédaction]

Introduction

Many pool-breeding mole salamanders of the genus *Ambystoma* produce aquatic larvae but inhabit terrestrial habitats as fossorial adults and juveniles. Although much research has focused on the larval ecology of pool-breeding *Ambystoma* spp. (e.g., Wilbur 1972; Wilbur and Collins 1973;

Semlitsch 1987; Pechmann et al. 1989), fewer studies have investigated their terrestrial ecology (e.g., Semlitsch 1981; Kleeberger and Werner 1983; Pechmann 1995; Windmiller 1996; Madison 1997). There is considerable evidence of density-dependent competition and predation affecting larvae, suggesting that population regulation may occur during the aquatic larval stage (e.g., Stenhouse et al. 1983; Wilbur

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and Collins 1973; Petranka 1989; Scott 1990; Van Buskirk and Smith 1991). This has led some researchers to suggest that competition for resources in the terrestrial environment may be limited and that, as a result, resource defense and territorial aggression may be weak or absent (Martin et al. 1986; Petranka 1989). However, factors affecting the spatial dispersion of *Ambystoma* spp. in terrestrial habitats remain poorly understood, and it is not known whether regulation sometimes occurs during the terrestrial juvenile or adult stages (Petranka 1989; Walls 1990; Van Buskirk and Smith 1991).

Many pool-breeding *Ambystoma* species migrate long distances to breed (the mean terrestrial migration distance was 125 m for six species; Semlitsch 1998) despite the fact that apparently suitable contiguous forest habitat often dominates the landscape surrounding breeding pools. This suggests that resource availability might limit terrestrial density (Windmiller 1996). Even if density-dependent effects within terrestrial habitats do not play a role in population regulation, the quality of terrestrial habitat may have significant fitness consequences for juveniles and adults. Therefore, a better understanding of the factors affecting terrestrial dispersion of pool-breeding *Ambystoma* species could contribute significantly to our understanding of their ecology and life-history evolution.

Spotted salamanders (*Ambystoma maculatum*) are rarely found above ground, often inhabit small-mammal burrows (Douglas and Monroe 1981; Kleeberger and Werner 1983; Madison 1997), and appear to be unable to excavate their own burrows (Semlitsch 1983). These findings suggest that spotted salamanders may be highly dependent upon small-mammal burrows as refugia. Several studies have documented intraspecific aggression that includes stereotyped displays, and in some experimental settings, spotted salamanders co-occupy burrows less frequently than expected by chance (Ducey and Ritsema 1988; Walls 1990; Ducey and Heuer 1991; Regosin et al. 2003a). Thus, it has been hypothesized that refuge availability affects the spatial dispersion of spotted salamanders in habitats surrounding breeding ponds, and that they may compete for high-quality refugia (Ducey and Ritsema 1988; Ducey 1989; Walls 1990). Fitness trade-offs between the costs associated with migration and (or) searching for refugia and the costs associated with poor refuge quality may account for observed migration distances and low terrestrial densities. Refuge quality may be a function of the physical characteristics of the refuge, or whether or not it is occupied by a conspecific or other species.

We used an experimental approach to investigate mechanisms that potentially influence the dispersion of *A. maculatum* in terrestrial habitats. First we used large-scale field enclosures to obtain data on the natural terrestrial densities of breeding spotted salamanders and then to conduct experimental manipulations of the densities of both small-mammal burrows and adult spotted salamanders. Our first experiment tested the hypothesis that removal of burrows is associated with an increased probability that salamanders would leave an area during the spring postbreeding migration, and thus that burrow availability can influence summer densities of salamanders. Our second experiment tested the hypothesis that a high density of spotted salamanders in terrestrial habitat is associated with decreased growth, fecundity, and probability of emerging to breed during subsequent breeding seasons.

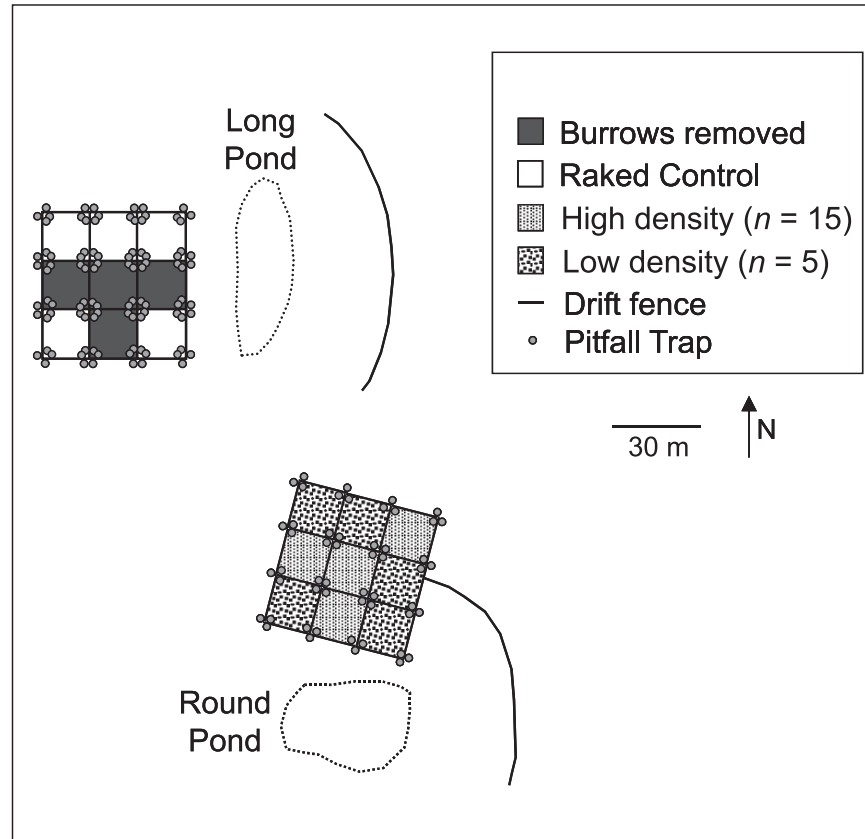
Methods

Enclosure construction and natural winter densities of salamanders

From 22 November to 8 December 1999, seventeen 16 × 17 m (272 m²) field enclosures (one array of eight and one of nine enclosures) were constructed in oak (*Quercus* spp.)-dominated upland deciduous forest adjacent to two spotted salamander breeding ponds in Sudbury, Massachusetts (42°21'N, 71°26'W) (Fig. 1). During spring 2000, one additional enclosure was added to the eight-enclosure array, to be used for the salamander density experiment. Enclosures were placed within blocks of well-drained upland forest habitat qualitatively similar to upland habitat located at other orientations to the breeding ponds, free of foot trails, and as close to the breeding ponds as possible while avoiding poorly drained soils. In addition, enclosures were located at compass orientations from the ponds through which a significant proportion of the spotted salamander population was known to have migrated in 1998 (B.S. Windmiller, unpublished data). Data on salamander migratory orientations in 1998 were obtained from pond-encircling drift fences that were removed prior to this study. Enclosures were constructed of 0.9 m high plastic silt fencing partially buried in a 25 cm deep trench. In the array of field enclosures used for the density manipulations, one 19-L pitfall trap was placed in each corner of each enclosure. Along the external walls of the enclosure array, two pitfall traps were placed at each corner. In addition, one pitfall trap was placed every 16–17 m along the external walls of the enclosure array at the point where the fences separating individual enclosures intersected with the external walls of the enclosure array (Fig. 1). The array used for the small-mammal-burrow experiment was set up similarly, except that two 19-L pitfall traps were placed in each corner of each enclosure with a 1 m long “runner” of silt fencing to separate them (Fig. 1). This design facilitated sorting the salamanders on the basis of their presumed direction of travel. All pitfall traps were lined with 25 × 25 cm sponges, which were kept damp throughout the study to prevent desiccation of the amphibians.

Although some movement of spotted salamanders occurs in our region into December, by late November they have generally arrived at wintering sites and are rarely captured moving on the surface during winter months (Madison 1997; J.V. Regosin and B.S. Windmiller, unpublished data). Pitfall traps were closed until 1 March 2000, prior to the spring prebreeding migration, which began on 11 March 2000. During the spring, traps were checked the day after every nighttime rain and at least once every 2–3 days, regardless of weather. Traps operated during the other three seasons for the experiments described below were checked after each nighttime rain and at least once every week, except when snowpack was present. Spotted salamanders captured emerging from the enclosures or at the external walls of the enclosures that were oriented toward a specific pond were weighed, sexed, measured (snout–vent length (SVL)), individually toe-clipped, and released directly into the pond toward which they were migrating. Salamanders captured moving laterally relative to a pond were released in their apparent direction of movement at the opposite end of the enclosure array. Thus, we were able to obtain data on the winter densities of spot-

Fig. 1. The Adams Road study site in Sudbury, Massachusetts. Spotted salamanders (*Ambystoma maculatum*) captured migrating toward either breeding pond at the outer wall of each enclosure array perpendicular to and farthest from the pond shore were assumed to have wintered >65 m from their breeding pond. Enclosures were assigned to experimental treatments as described in the text. Pitfall traps are not drawn to scale. Additional drift fencing on the east side of each pond was operated only during the postbreeding migration to obtain salamanders for the density experiment (pitfall traps are not shown). Number of salamanders per enclosure (n) is given in the legend.



ted salamanders in 17 enclosures adjacent to two breeding ponds. In addition, it was possible to compare the characteristics of animals wintering within the enclosures, <65 m from the breeding ponds, with those of animals captured in the spring at the outer wall of each enclosure farthest from, and roughly parallel to, the bank of the breeding pond, indicating that they had wintered at greater distances from their breeding ponds.

Small-mammal-burrow experiment

The probability of salamanders remaining in a given enclosure may be a function of distance from the breeding pond and habitat quality, independent of burrow density. As a result, the following procedure was used to assign enclosures to control and experimental treatments. We reasoned that variation in winter density of salamanders might reflect variation in habitat quality among enclosures. Therefore, enclosures were first ranked in order of natural winter density of breeding salamanders and then paired in descending rank order. One member of each pair was selected randomly as the experimental (burrow-removal) enclosure. This method was restricted to require at least one control and one experimental plot for each of the three rows of enclosures parallel to the pond shore (each row was a distance class). The odd

ninth enclosure was assigned to the control treatment, resulting in five control and four experimental plots.

Throughout March and early April of each year, small mammals were captured in pitfall traps and removed from the enclosure array. Beginning on 7 April 2000, after several nighttime rains during which no new salamanders emerged from the enclosures to breed, leaf litter was systematically removed and then restored within each enclosure. In the experimental enclosures, small-mammal burrows were counted and sealed firmly with dirt prior to restoring the leaf litter. This procedure was repeated during spring 2001 after all breeding individuals had been removed from the enclosures. At this time, one control enclosure was accidentally converted to a burrow-removal plot. As a result, when data were pooled across years in analyses (see below), 2001 data from this enclosure were excluded from analysis. In each year, salamanders leaving the breeding ponds were captured at the external walls of the enclosure array before burrow removal was completed. These salamanders were individually toe-clipped, held in small plastic bags or containers in a refrigerator for up to 25 days prior to enclosure manipulations, and then released into the enclosures opposite their points of capture (first release dates were 22 and 25 April in 2000 and 2001, respectively). As salamanders entered or moved through

the enclosure array, they were captured in pitfall traps, individually toe-clipped if they had not been previously captured, and placed across (i.e., on the other side of) the enclosure walls in their apparent direction of movement.

Pitfall traps were operated continuously from March 2000 to March 2002, and additional salamanders entering the array were processed as described above. Salamanders exhibited a bimodal distribution of movement and capture, peaking in the spring, with a smaller peak of activity in the fall (see Windmiller 1996). During the 2 years of the study, only two salamanders were captured from 13 June to 13 September. Thus, animals that did not leave the enclosures prior to the summer inactive period could be classified as summer residents, and the proportions of animals that left the experimental and control enclosures prior to the onset of the summer inactive period could be compared. Inspection and resealing of burrows during the spring of 2001, prior to the second year of the experiment, indicated that some small mammals had gained entry to experimental plots and re-excavated burrows. Thus, while we were confident that we had greatly reduced burrow availability during the 6–8 weeks between burrow removal and the onset of the summer inactive period, burrow availability within the experimental enclosures may have increased by the following fall. Therefore, no attempt was made to analyze fall movement patterns.

Two approaches were used to test for effects of burrow removal on salamander movement patterns. First, backward stepwise multiple regression ($p < 0.05$ to enter, $p > 0.10$ to remove) was used to test for effects of treatment and two covariates on the proportion of entering salamanders that subsequently left a given enclosure. The covariates were the proportion of females entering an enclosure (to control for gender differences in probability of movement) and distance from the breeding pond (on a three-point scale for the three rows of enclosures located parallel to the pond shore). This approach treats the enclosure as the unit of analysis. An alternative approach is to explicitly model, and statistically control for, the covariance among multiple within-subject movement “decisions” as individuals move through the enclosure array by using generalized estimating equations (GEEs) (Liang and Zeger 1986). Using this approach, a repeated-measures logistic model was constructed to evaluate the effects of treatment, gender, and distance to the breeding pond on the probability of leaving an enclosure. Within-subject covariance among movement decisions (to stay in or leave a particular enclosure) was modeled as exchangeable, and empirical standard error estimates were used to determine p values for the parameters (SAS Institute Inc. 1999). GEEs are a powerful tool for analyzing correlated response data, and have recently been applied to ecological data (Brand and George 2001; Johnson and Igl 2001; McLaughlin 2001; Regosin et al. 2003a).

Density experiment

During April 2000, after the prebreeding migration, each enclosure was populated with spotted salamanders at either high (9 males, 6 females) or low (3 males, 2 females) density. The low density was somewhat higher than the mean natural winter density of salamanders observed within the enclosures (mean = 2.7/enclosure, SD = 3.0/enclosure, range

0–11/enclosure, $n = 17$; see below), while the high density was close to the high end of the range of natural densities observed by Windmiller (1996) at a nearby breeding pond. The male-biased sex ratio simulates the male-biased sex ratios of breeding populations (Petranka 1998). As spotted salamanders sometimes skip breeding attempts (Husting 1965; Homan 2003), some resident salamanders may have remained in the enclosures prior to initiation of the experiment, slightly altering the experimental densities. However, year-skipping in this study appeared to be infrequent, as it was in a nearby pond (Homan 2003). Over the course of the 2-year study, only 7 animals that were not previously toe-clipped were captured within the enclosures, indicating possible skipping of breeding attempts (these individuals were removed upon capture). As these animals were spread out across five enclosures, the presence of uncaptured resident salamanders did not have an appreciable effect on experimental densities. Since habitat quality may have varied among enclosures, and habitat quality may have affected salamander growth and survivorship independently of density, a random stratification based upon observed winter densities was used in assigning enclosures to the high- and low-density treatments ($n = 4$ and 5 , respectively) as described above for the small-mammal-burrow experiment. However, the natural winter density of salamanders in the single enclosure constructed in the spring was not known, and this enclosure was assigned to the low-density treatment.

During early April, postbreeding spotted salamanders were captured at additional pitfall-trap and drift-fence arrays located on the eastern side of each breeding pond (Fig. 1). Salamanders were captured in these areas to avoid removing animals that were likely to enter the enclosure array used for the small-mammal-burrow experiment. Captured salamanders were weighed, measured, individually toe-clipped, randomly assigned to enclosures (to meet sex-ratio quotas), and released. Three of the four pitfall traps within each enclosure were closed. The remaining pitfall trap was left open to allow other amphibian species to emigrate. Each fall, from mid-October to early December, a period of increased spotted salamander movement, all four pitfall traps in each enclosure were reopened. Captured salamanders were released back into their enclosures, and capture rates were used to calculate indices designed to reflect dispersal tendency during both the fall and the previous spring postbreeding period. These indices were (i) the proportion of individuals “dispersing”, defined as the proportion of individuals introduced into an enclosure that were captured attempting to leave it; (ii) the number of captures (dispersal attempts) for each individual attempting to disperse; and (iii) the proportion of individuals in an enclosure that repeatedly attempted to disperse. Traps were reopened in March, prior to the breeding migration. Each spring, salamanders captured emerging from enclosures were placed in 1.3 m diameter artificial pools filled with pond water (20 cm deep), leaf litter, and submerged twigs. Salamanders from each treatment, as well as those that trespassed across treatments, were bred in separate artificial pools. Egg masses in each pool were counted every few days, and after 4–6 days with no new egg masses appearing, salamanders were removed from the breeding pools, weighed, and, in the first year of the study, returned to their assigned enclosures.

Salamanders that failed to emerge to breed may have died, escaped, or skipped a breeding opportunity. Therefore, during spring 2001, additional salamanders were captured emerging from the breeding ponds and placed in the enclosures to replace any that failed to emerge to breed. Because some animals that failed to breed were still present in the enclosures, the exact densities of salamanders within the enclosures during the second year of the study are not known. The experiment was terminated after the breeding migration in spring 2002. Throughout the study period, salamanders that trespassed through the fencing and were captured in another enclosure were returned to their assigned enclosure.

All proportions were arcsine-transformed prior to analysis. Before performing Student's *t* tests we tested for homogeneity of variances, and when this assumption was not met, an approximate Satterthwaite *t* value was used, based upon the assumption of unequal variances (Satterthwaite 1946; SAS Institute Inc. 1999). Experiments were conducted in accordance with the principles and guidelines of the Canadian Council on Animal Care.

Results

Natural winter densities of salamanders

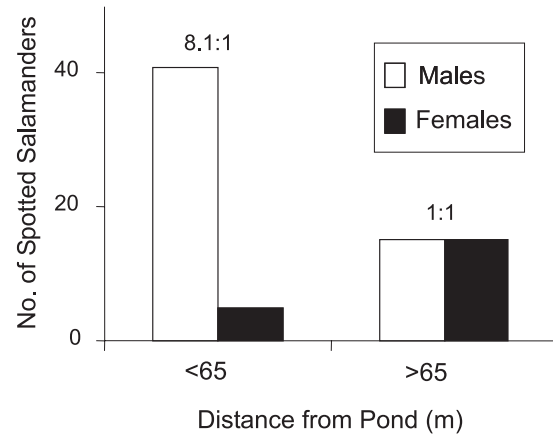
Spotted salamanders migrated to the breeding ponds from 11 March to 4 April. Forty-one male and 5 female salamanders were captured emigrating from the enclosures, 85% of them during the rainy night of 10–11 March. The mean capture date was significantly earlier for males (12 March) than for females (17 March; Satterthwaite *t* test, $t = 2.79$, $df = 24.1$, $p < 0.01$), including animals captured both within and at the outer walls of the enclosures. The sex ratio was skewed significantly toward males (8.2:1) among animals within the enclosures but not among animals captured at the outer wall of each enclosure array running roughly parallel to the pond shore, located approximately 65 m from the breeding ponds (1:1; $\chi^2 = 14.3$, $df = 1$, $p < 0.001$) (Fig. 2). The mean density of salamanders within the enclosures was 2.7 (SD = 3.0, $n = 17$), or 1.0/100 m² (range = 0–4.0/100 m²). However, since the sex ratio was highly skewed, the mean density of females within the enclosure system was only 0.3 (SD = 0.5, $n = 17$), or 0.1/100 m². Multiple regression analysis indicated that the salamander density within the enclosures was negatively related to the distance to the nearest breeding pond ($t = 2.49$, $df = 1$, $p < 0.03$) (Fig. 3).

Males residing within the enclosures weighed more than males wintering at greater distances from the breeding ponds ($t = 1.97$, $df = 54$, $p = 0.055$) (Table 1). Among males, SVL did not differ by distance from the pond ($t = 1.14$, $df = 54$, $p = 0.26$), and among females there were no mean differences in either morphological trait (body mass: $t = 0.04$, $df = 18$, $p = 0.97$; SVL: $t = 0.16$, $df = 13$, $p = 0.88$) (Table 1), although analysis was hindered by a small sample of females residing within the enclosures.

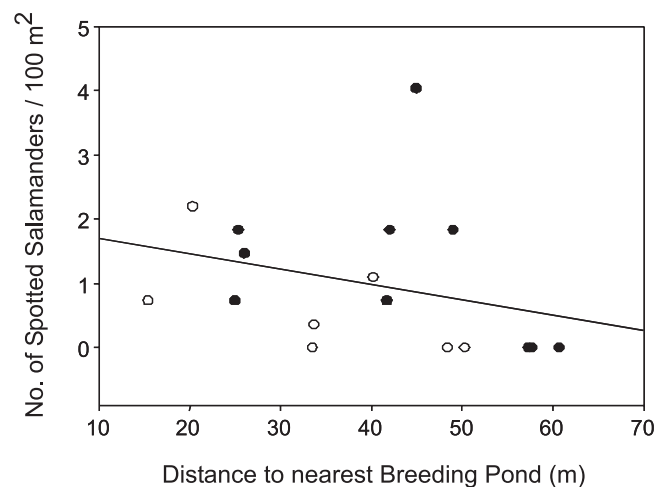
Small-mammal-burrow experiment

The mean density of small-mammal burrows within burrow-removal plots was 80.0/100 m² (SD = 28.1/100 m², $n = 4$). Twenty-eight salamanders entered the enclosure array during spring 2000, but only 16 entered during spring 2001, a season in which a late-melting snowpack and few rainy nights

may have resulted in low numbers of breeders across the region. The mean proportion of salamanders leaving the enclosures (Fig. 4) was significantly higher for treatment enclosures (burrows removed) than for controls in spring 2000, or for spring 2000 and spring 2001 combined (Table 2). Some salamanders that were not detected leaving enclosures may have escaped, and because cells differed in the number of walls bordering other enclosures (where trespass would be detected) versus the number of external walls (where trespass would not be detected), this could have confounded the observed treatment effect. Consequently, we redid our 2000–2001 analysis in an attempt to compensate for this possibility. The observed trespass rate among enclosures (the proportion of captures in enclosures other than the en-



densities of wintering spotted salamanders emerging from enclosures in spring 2000 versus distance to the nearest breeding pond. Ponds are those shown in Fig. 1. Multiple regression analysis of square-root-transformed densities indicate a significant effect of distance to the nearest breeding pond ($p < 0.03$). A pond effect approached significance ($p < 0.07$), with generally higher densities associated with enclosures near Long Pond (model: $F_{[2,14]} = 3.91$, $p < 0.05$, adjusted $R^2 = 0.27$).



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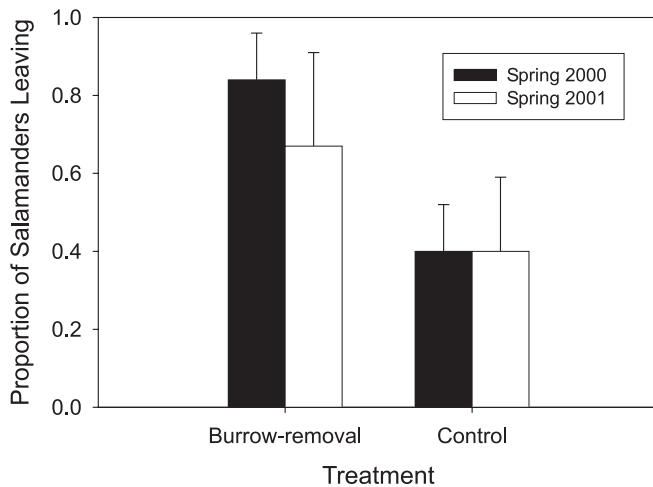
Table 1. Morphological measurements of male and female spotted salamanders (*Ambystoma maculatum*) wintering within enclosures <65 m from their breeding pond and outside enclosures >65 m from their breeding pond.

	Males		Females	
	<65 m	>65 m	<65 m	>65 m
Body mass (g)	10.0 (2.5, 41)	8.6 (2.3, 15) ^a	17.5 (4.6, 5)	17.5 (2.9, 15)
Snout–vent length (mm)	77.8 (5.8, 41)	75.7 (7.2, 15)	91.5 (6.6, 5)	91.1 (4.2, 10)

Note: Values are given as the mean with SD followed by the sample size in parentheses.

^a $p < 0.06$ (t test).

Fig. 4. Proportions (mean + SE) of spotted salamanders leaving burrow-removal and control enclosures in spring 2000–2001. There were four burrow-removal plots in 2000 and five in 2001, out of a total of nine enclosures (see the text for details). Mean differences between treatments were significant in 2000 but not in 2001.



closure of release) was 10.2%, although if trespassers detected the following fall are included (these individuals may have trespassed in either fall or spring), the trespass rate may have been as high as 14.1%. To be conservative, we used the latter value to estimate a trespass rate per enclosure wall, assuming that trespass was equally likely across any wall. We used this value and the number of external walls (external to the study enclosures) to estimate the rate of escape of salamanders from each enclosure, and adjusted the estimated proportion of salamanders leaving each enclosure accordingly. This adjusted analysis still indicated a treatment effect that approached statistical significance ($p = 0.052$).

The repeated-measures GEE logistic model for the 2000–2001 data produced similar results: salamanders were estimated to be 3.3 times more likely to leave burrow-removal versus control plots when distance from the breeding pond and within-subject correlations among movement decisions were controlled for (95% CI for odds ratio = 1.2–9.1, $p = 0.01$). In addition, salamanders were less likely to leave enclosures as the distance from the breeding pond increased (95% CI for odds ratio = 0.21–0.66, $p = 0.001$). To account for potential escaping salamanders, the GEE analysis was performed again, including only those animals that were confirmed, through recapture the following fall or spring, to have remained in a given enclosure. The treatment effect still

Table 2. Reduced model results from stepwise multiple regression analyses of the effects of treatment (removal of small-mammal burrows), distance from the breeding pond, number of spotted salamanders initially residing in each enclosure, and proportion of females entering the enclosures on the proportion (arcsine-transformed) of spotted salamanders leaving the enclosures ($n = 9$).

Model	t	p	Model F	p
Spring 2000				
Treatment (control)	2.89	0.012	8.34	0.023
Spring 2001				
Treatment (control)	1.42	0.102	5.91	0.038
Distance	3.13	0.020		
Spring 2000–2001				
Treatment (control)	2.59	0.021	8.48	0.018
Distance	3.21	0.019		

Note: The p values for treatment are one-tailed, while all other p values are two-tailed. When data were pooled across years, data from individuals entering the enclosure array in both years was included for 2000 only. For 2001, treatment was forced into the model, as it did not enter through the stepwise procedure.

approached statistical significance ($p < 0.06$). This analysis is conservative, as traps were not operated in spring 2002, and spotted salamanders sometimes skip years between breeding attempts (Husting 1965). As a result, additional salamanders recorded as remaining in the enclosures but not recaptured were likely to have still been present in the enclosures.

Density experiment

On average, 22% of high-density (SD = 5.8%, $n = 4$) and 50% of low-density (SD = 21.5%, $n = 5$) salamanders emerged within their enclosures (apparently to breed) either 1 or 2 years after being placed in the system (Fig. 5; one-tailed t test on arcsine-transformed proportions, Satterthwaite $t = 2.54$, $df = 4.5$, $p = 0.029$). We found no gender ($\chi^2 = 0.40$, $df = 1$, $p = 0.53$) or year ($\chi^2 = 0.42$, $df = 1$, $p = 0.52$) differences in probability of emerging from enclosures during the spring breeding season. Salamanders that trespassed to a neighboring enclosure for the first fall or spring prebreeding capture were excluded from analysis, as these individuals may have spent considerable time out of their experimental treatments. The difference in proportions of salamanders emerging remains significant when data from animals released in 2000 are analyzed separately ($t = 2.65$, $df = 7$, one-tailed test, $p = 0.017$). Because the number of new animals placed in the enclosures during spring 2001 varied, and was sometimes quite small (see Methods), data from 2001 animals were not

Table 3. Dispersal indices reflecting the tendency of spotted salamanders to leave high-density ($n = 4$) and low-density ($n = 5$) enclosures.

	High-density treatment	Low-density treatment	p^a
Proportion that attempted to disperse ^b			
2000	0.58 (0.19)	0.48 (0.27)	0.284
2000–2001	0.66 (0.07)	0.56 (0.17)	0.155
No. of dispersal attempts per disperser			
2000	1.88 (0.23)	1.25 (0.43)	0.018
2000–2001	1.88 (0.22)	1.58 (0.46)	0.133
Proportion that repeatedly attempted to disperse			
2000	0.25 (0.08)	0.12 (0.18)	0.118
2000–2001	0.32 (0.03)	0.22 (0.21)	0.212
Proportion that trespassed ^c			
2000–2001	0.40 (0.40)	0.12 (0.18)	0.203
2000–2002	0.37 (0.34)	0.13 (0.09)	0.166

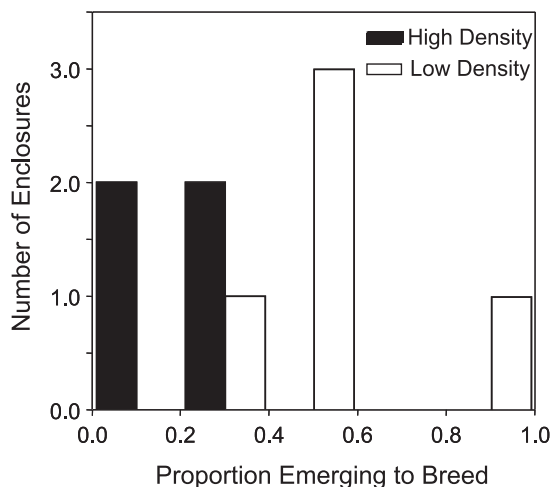
Note: The indices, given as the mean with SD in parentheses, measure movement activity during the fall and during the spring postbreeding period. Data from the second year of the study include only the movement behavior of animals newly placed in the enclosures in that year; t tests were performed on arcsine-transformed proportions. The mean value for the enclosure is the unit of analysis.

^aAll p values are one-tailed, except for differences in the trespass rate, for which there was no a priori hypothesis.

^bThe proportion of individuals captured while attempting to leave an enclosure.

^cThe proportion of animals captured in enclosures other than the enclosure of release, through the breeding seasons of 2001 and 2002.

Fig. 5. Frequency distribution of proportions of spotted salamanders from high-density (5.5 salamanders/100m²) and low-density (1.8 salamanders/100m²) enclosures that re-emerged from the enclosures to breed at least once during the 2-year study. Salamanders that trespassed into a neighboring enclosure during the first fall capture or the first spring prebreeding capture were excluded from analysis. Salamanders introduced into enclosures in both 2000 and 2001 were included in the analysis, as there was no significant difference between years in the proportion of salamanders that re-emerged to breed.



analyzed separately. We also performed a nested logistic regression to test for a treatment effect on probability of emerging, controlling for the effect of enclosure nested within treatment. This analysis also indicated a significant treatment effect, with salamanders from the low-density treatment estimated to be 2.3 times more likely to emerge than salamanders from the high-density treatment ($\chi^2 = 4.17$, $df = 1$, $p = 0.021$). On average, 22% (SD = 17.9%) of the

non-trespassing salamanders introduced into the five low-density enclosures during spring 2000 emerged to breed in both years of the experiment, compared with 8.0% (SD = 11.8%) of the individuals from the four high-density enclosures, although the difference was not statistically significant (arcsine-transformed data, one-tailed test, $t = 1.34$, $df = 7$, $p = 0.11$).

Although the number of external walls (those not abutting adjacent enclosures) did not differ between treatments ($t = 0.51$, $df = 7$, $p = 0.63$), there was a nonsignificant trend toward higher trespass rates in the high-density treatment (Table 3). If high trespass rates were indicative of non-escape-proof enclosures, then a higher escape rate could account for observed differences in proportion emerging across treatments. In an attempt to address this issue, we adjusted the proportion of salamanders emerging from each enclosure to account for escaping salamanders, based upon the observed treatment-specific trespass rates (low density, 0.05/wall; high density, 0.12/wall), as well as the number of external walls per enclosure (as described above). The analysis of adjusted data still indicated a significant treatment effect (Satterthwaite, $t = 2.36$, $df = 4.3$, one-tailed test, $p = 0.037$).

We also analyzed the capture rates of salamanders during the spring postbreeding and fall active periods in an effort to compare dispersal tendencies between treatments. There were no differences between treatments in the proportion of individuals recaptured or in the percentage of individuals recaptured repeatedly. However, among recaptured individuals, the mean number of dispersal attempts was significantly higher in the high-density treatment during 2000 (Table 3).

Although we were hindered by small sample sizes, we performed nested analyses of variance (enclosure nested within treatment) to test for treatment effects on change in body mass and change in SVL among animals recaptured during the fall or during the spring breeding period. We used the same approach to test for differences in female mass loss

during breeding (likely to be correlated with clutch size) across treatments. In cases where SVL was correlated with the response variable, SVL was included as a covariate in the model. None of these analyses showed significant treatment differences ($p > 0.10$ for treatment effect, all models).

Discussion

Observed winter densities of spotted salamanders in well-drained upland deciduous habitat adjacent to the breeding ponds were generally low (0–4/100 m²), and similar to those reported by Windmiller (1996). Densities within enclosures declined as distance to the breeding pond increased, although enclosures were contiguous and thus not independent sampling units. Our enclosures were sited in areas through which large numbers of salamanders were known to have migrated during a previous breeding season (B.S. Windmiller, unpublished data), and the oak (*Quercus* spp.)-dominated forest within enclosures was qualitatively similar to habitat available at other compass orientations. Nonetheless, amphibians often enter and leave breeding sites in nonrandom patterns (Dodd and Cade 1998), so additional research is needed to confirm that observed densities were typical of densities at other compass orientations to the breeding ponds.

We believe that our estimates of winter density are reasonably accurate, although some salamanders may have trespassed across the drift fences during the spring migration (cf. Dodd and Scott 1994). Research at a nearby site with multiple drift fences and individually marked salamanders suggests that trespass rates were likely to have been between 10 and 20% (Windmiller 1996). If this was the case, then trespass could have resulted in a modest overestimate of salamander densities within enclosures, because more salamanders were captured at the outer walls of the enclosures than emerging from within the enclosures.

Zweifel (1989) hypothesized that male wood frogs (*Rana sylvatica*) might gain a fitness advantage by hibernating close to the breeding pond, as this might enable them to arrive to breed earlier in the spring (also see Regosin et al. 2003b). This may be true for spotted salamanders as well, and our findings of a highly male-biased sex ratio, and heavier males <65 m from the breeding pond are consistent with this hypothesis. Our results also suggest that, on average, females winter farther from the breeding pond than do males. Salamanders captured at the outer wall of each enclosure array located roughly parallel to the pond shore were considered to have wintered >65 m from their breeding pond. Although these individuals could have wintered closer to the breeding pond if they had first moved directly away from the pond and then abruptly reversed direction, our data from another site indicate that this is unlikely. At a nearby site with concentric rings of drift fencing located 0, 60, 100, and 200 m from a breeding pond, it was very rare for salamanders to be captured moving away from the breeding pond during the prebreeding migration (J.V. Regosin and B.S. Windmiller, unpublished data).

We also found that salamanders were more likely to leave and less likely to spend the summer within enclosures from which small-mammal burrows had been removed. This is the first reported experimental manipulation of spotted salamander habitat in order to influence salamander densities, and

the results are consistent with prior research indicating that small-mammal burrows are important underground refugia (Douglas and Monroe 1981; Kleeberger and Werner 1983; Windmiller 1996; Madison 1997). Although our results are consistent with the hypothesis that the availability of small-mammal burrows might limit spotted salamander densities within terrestrial habitats, it is unclear whether densities of naturally occurring burrows are low enough to actually be limiting. Although burrows at our site appeared to be abundant (mean = 80/100m²), burrow quality might vary considerably, and high-quality burrows might be scarce (Ducey and Ritsema 1989). For example, we observed few burrows that appeared to penetrate deeply into the mineral soil, and there is some indication that spotted salamanders tend to inhabit deeper burrows, particularly in winter (Madison 1997; B.S. Windmiller, unpublished data). An experimental increase in the density of small-mammal burrows could be used to directly test the hypothesis that burrow availability limits salamander densities, although such an experiment might be logistically difficult.

Our observation that spotted salamanders housed at high density were less likely to re-emerge in subsequent years to breed than were salamanders housed at low density suggests that density-dependent competition could occur in terrestrial habitats. This result, however, could be due to a systematic bias in escape rates, as suggested by the higher (but not statistically significant) trespass rates from the high-density enclosures. This bias could indicate that enclosures were less escape-proof (experimental error) or that animals in high-density enclosures had an increased motivation to escape. Treating the difference as a potential source of error, we addressed this issue to the best of our ability by adjusting observed re-emergence rates on the basis of treatment-specific trespass rates. However, these adjustments assumed that trespass (or escape) was equally likely across each enclosure wall, and this may not have been the case. Therefore, more research is needed to provide support for our finding of density-dependence within the terrestrial environment.

Although prior research has focused on the role of the breeding pond in influencing the population dynamics of *Ambystoma* spp. (e.g., Wilbur and Collins 1973; Stenhouse et al. 1983; Petranka 1989; Scott 1990; Van Buskirk and Smith 1991), studies of conspecific avoidance and aggression suggest that conspecific interactions could influence terrestrial dispersion (Ducey and Ritsema 1988; Ducey 1989; Walls 1990; Ducey and Heuer 1991). This study provides additional evidence that the distribution of conspecifics and burrow availability might play roles in limiting the density of spotted salamanders within terrestrial habitats, and suggests that intraspecific competition in terrestrial habitat could, in some cases, regulate *Ambystoma* spp. populations (Windmiller 1996). We hypothesize that resource availability within terrestrial habitats could play a variety of roles in shaping the ecology and life-history evolution of pool-breeding *Ambystoma* spp. For example, trade-offs between habitat-quality variation, competition, and migration distance could affect age at first reproduction, fecundity, and the frequency of breeding attempts (Husting 1965; Bull and Shine 1979; Scott 1990).

Our results may have a number of significant conservation implications for spotted salamanders, which do not inhabit

open habitats and are sensitive to forest loss and forest edges (deMaynadier and Hunter 1998; Gibbs 1998). First, given the low observed densities, relatively few spotted salamanders would be protected within narrow (15–30 m) regulated terrestrial buffer zones surrounding breeding ponds, and the overwhelming majority (perhaps around 85%) of these animals may be male (Semlitsch 1998). Habitat loss that disproportionately impacts females could have a particularly dramatic impact on the risk of local extinction. Second, spotted salamanders may be sensitive to changes in the density of burrowing mammals, and this may lead to unforeseen indirect effects of habitat fragmentation and land-management regimes on populations of spotted salamanders and other *Ambystoma* species. Finally, although it remains unclear whether, and under what conditions, terrestrial-habitat availability might limit pool-breeding *Ambystoma* spp. populations, our results suggest that terrestrial-habitat loss, in addition to causing direct mortality, could increase the intensity of competition, and thereby directly affect local population dynamics and extinction risks.

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References

- Brand, L.A., and George, T.L. 2001. Response of passerine birds to forest edge in coast redwood forest fragments. *Auk*, **118**: 678–686.
- Bull, J.J., and Shine, R. 1979. Iteroparous animals that skip opportunities for reproduction. *Am. Nat.* **114**: 296–303.
- deMaynadier, P.G., and Hunter, M.L., Jr. 1998. Effects of silvicultural edges on the distribution and abundance of amphibians in Maine. *Conserv. Biol.* **12**: 340–352.
- Dodd, C.K., Jr., and Cade, B.S. 1998. Movement patterns and the conservation of amphibians breeding in small, temporary wetlands. *Conserv. Biol.* **12**: 331–339.
- Dodd, C.K., Jr., and Scott, D.E. 1994. Drift fences encircling breeding sites. In *Measuring and monitoring biological diversity: standard methods for amphibians*. Edited by W.R. Heyer, M.A. Donnelly, R.W. McDiarmid, L.C. Hayek, and M.S. Foster. Smithsonian Institution Press, Washington, D.C. pp. 125–130.
- Douglas, M.E., and Monroe, B.L., Jr. 1981. A comparative study of topographical orientation in *Ambystoma* (Amphibia: Caudata). *Copeia*, 1981: 460–463.
- Ducey, P.K. 1989. Agonistic behavior and biting during intraspecific encounters in *Ambystoma* salamanders. *Herpetologica*, **45**: 155–160.
- Ducey, P.K., and Heuer, J. 1991. Effects of food availability on intraspecific aggression in salamanders of the genus *Ambystoma*. *Can. J. Zool.* **69**: 288–290.
- Ducey, P.K., and Ritsema, P. 1988. Intraspecific aggression and response to marked substrates in *Ambystoma maculatum*. *Copeia*, 1988: 1008–1013.
- Gibbs, J.P. 1998. Distribution of woodland amphibians along a forest fragmentation gradient. *Landsc. Ecol.* **13**: 263–268.
- Homan, R.N. 2003. Conservation studies of amphibian health at individual, population, and landscape scales. Ph.D. dissertation, Tufts University, Medford, Mass.
- Husting, E.L. 1965. Survival and breeding structure in a population of *Ambystoma maculatum*. *Copeia*, 1965: 352–362.
- Johnson, D.H., and Igl, L.D. 2001. Area requirements of grassland birds: a regional perspective. *Auk*, **118**: 24–34.
- Kleeberger, S.R., and Werner, J.K. 1983. Post-breeding migration and summer movement of *Ambystoma maculatum*. *J. Herpetol.* **17**: 176–177.
- Liang, K.Y., and Zeger, S.L. 1986. Longitudinal data analysis using generalized linear models. *Biometrika*, **73**: 13–22.
- Madison, D.M. 1997. The emigration of radio-implanted spotted salamanders, *Ambystoma maculatum*. *J. Herpetol.* **31**: 542–552.
- Martin, D.L., Jaeger, R.G., and Labat, C.P. 1986. Territoriality in an *Ambystoma* salamander? Support for the null hypothesis. *Copeia*, 1986: 725–731.
- McLaughlin, R.L. 2001. Behavioral diversification of brook charr: adaptive responses to local conditions. *J. Animal Ecol.* **70**: 325–337.
- Pechmann, J.H.K. 1995. Use of large field enclosures to study the terrestrial ecology of pond-breeding amphibians. *Herpetologica*, **51**: 434–450.
- Pechmann, J.H.K., Scott, D.E., Semlitsch, R.D., and Gibbons, J.W. 1989. Influence of wetland hydroperiod on diversity and abundance of metamorphosing juvenile amphibians. *Wetlands Ecol. Manag.* **1**: 1–9.
- Petranka, J.W. 1989. Density-dependent growth and survival of larval *Ambystoma*: evidence from whole-pond manipulations. *Ecology*, **70**: 1752–1767.
- Petranka, J.W. 1998. Salamanders of the United States and Canada. Smithsonian Institution Press, Washington, D.C.
- Regosin, J.V., Windmiller, B.S., and Reed, J.M. 2003a. Effects of conspecifics on the burrow occupancy behavior of spotted salamanders (*Ambystoma maculatum*). *Copeia*, 2003. In press.
- Regosin, J.V., Windmiller, B.S., and Reed, J.M. 2003b. Terrestrial habitat use and winter densities of the wood frog (*Rana sylvatica*). *J. Herpetol.* **37**. In press.
- SAS Institute Inc. 1999. SAS OnlineDoc®. Version 8. SAS Institute Inc., Cary, N.C.
- Satterthwaite, F.E. 1946. An approximate distribution of estimates of variance components. *Biom. Bull.* **2**: 110–114.
- 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. 1981. Terrestrial activity and summer home range of the mole salamander (*Ambystoma talpoideum*). *Can. J. Zool.* **59**: 315–322.
- Semlitsch, R.D. 1983. Burrowing ability and behavior of salamanders of the genus *Ambystoma*. *Can. J. Zool.* **61**: 616–620.
- Semlitsch, R.D. 1987. Relationship of pond drying to the reproductive success of the salamander *Ambystoma talpoideum*. *Copeia*, 1987: 61–69.
- Semlitsch, R.D. 1998. Biological delineation of terrestrial buffer zones for pond-breeding salamanders. *Conserv. Biol.* **12**: 1113–1119.
- Stenhouse, S.L., Hairston, N.G., and Cobey, A.E. 1983. Predation and competition in *Ambystoma* larvae: field and laboratory experiments. *J. Herpetol.* **17**: 210–220.
- Van Buskirk, J., and Smith, D.C. 1991. Density-dependent population regulation in a salamander. *Ecology*, **72**: 1747–1756.

- Walls, S.C. 1990. Interference competition in postmetamorphic salamanders: interspecific differences in aggression by coexisting species. *Ecology*, **7**: 307–314.
- Wilbur, H.M. 1972. Competition, predation, and structure of the *Ambystoma* – *Rana sylvatica* community. *Ecology*, **53**: 3–21.
- Wilbur, H.M., and Collins, J.P. 1973. Ecological aspects of amphibian metamorphosis. *Science* (Wash., D.C.), **182**: 1305–1314.
- Windmiller, B.W. 1996. The pond, the forest, and the city: spotted salamander ecology and conservation in a human-dominated landscape. Ph.D. dissertation, Tufts University, Medford, Mass.
- Zweifel, R.G. 1989. Calling by the frog, *Rana sylvatica*, outside the breeding season. *J. Herpetol.* **23**: 185–186.