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Orientation and Sexual Differences During Breeding Migrations of the Spotted Salamander, *Ambystoma maculatum*

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We conducted a 2 yr drift fence study of individually marked *Ambystoma maculatum* at a woodland pond in east-central Missouri. In 1985–86, 24% and 45% of the marked animals exited the pond within one drop can (5 m) of their respective entry point. In 1986, 35% of the marked salamanders entered within one drop can of their 1985 entry point. These results are strikingly similar to those of other studies of *A. maculatum*. No difference in orientation ability could be detected between the sexes. During two breeding seasons, the sex ratio of marked individuals remained the same.

DURING the past several decades, a number of investigators have studied the migration of ambystomatid salamanders between their terrestrial habitat and their breeding ponds. Several of these studies demonstrate that individuals use the same route when moving between the two habitats both within and among years (Shoop, 1965; Hardy and Raymond, 1980;

Douglas and Monroe, 1981; Stenhouse, 1985; Beneski et al., 1986). This ability, hereafter referred to as migratory orientation, should be advantageous because it allows the salamanders to locate a secure breeding habitat (Grubb, 1973; Gill, 1978; Hedgecock, 1978) and home range (Stenhouse, 1985).

Our overall objective was to extend the investigation of migratory orientation to include *Ambystoma maculatum* in east-central Missouri. Novel contributions of our study include; 1) comparison of our results with previous studies; 2) a test of the hypothesis that males and females of this species do not differ in their orientation abilities; and 3) a test of two possible explanations for the male-biased sex ratio in *A. maculatum*: differential mortality between the sexes (Husting, 1965; Whitford and Vinegar, 1966) and the failure of females to breed in consecutive years (Husting, 1965).

Materials and Methods

The breeding pond is located in a second growth oak-hickory forest on the Tyson Research Center of Washington University in St. Louis County, Missouri (Sexton et al., 1986). A 0.64 cm mesh hardware cloth drift fence partially enclosed the pond in 1985 and completely enclosed it in 1986. The completed fence was 150 m in circumference, 30–50 cm high, and from 1–14 m from the edge of the pond at high water. Twenty-six pairs of five liter plastic buckets were buried every 5–6 m on both sides of the fence. We checked the fence daily during the spring breeding season, 21 Feb.–21 April, 1985 and 1 Feb.–30 April 1986 and individually marked a portion of the total immigrant salamanders by suturing a unique series of glass beads to the tail. This procedure required that the animals be transported to a nearby (1 km) facility and anesthesized with ethyl-m-aminobenzoate methanesulfonate. We seldom held animals for more than 4 h. We recorded the drop-can number, sex, and date for each salamander and then released them in the pond. Upon subsequent recaptures in the same season, similar data were recorded and the salamanders were released outside of the fence. We also recorded the same data for all unmarked salamanders during both breeding seasons.

We used the distance between entry and exit drop cans within each year and the distance between entry in 1985 and entry in 1986 as measures of orientation ability. Animals encountered entering or leaving the fenced area more than once in one breeding season were excluded from the analysis. Entry/exit and entry/entry are hereafter refered to as fence encounters.

The mean number of drop cans between fence encounters, $\bar{x}_o$, and the mean number expected if fence encounters were random with respect to each other, $\bar{x}_r$, were calculated according to Shoop and Doty (1972). We tested the null hypothesis $\bar{x}_o = \bar{x}_r$ using a t-test. For the completed drift fence in this study $\bar{x}_r = 6.5$ drop cans. However, for 1985, when the fence was incomplete, we could not calculate $\bar{x}_r$ and hence used a chi-square for hypothesis testing. We also calculated the percentage of salamanders that had a distance between fence encounters of one drop can or less. Comparisons were made between the sexes by testing the hypothesis that $\bar{x}_{o\delta} = \bar{x}_{o\varphi}$ using the t-test.

We obtained the number of degrees between fence encounters using a reference point of zero degrees for the first encounter. These data were analyzed using the circular statistical methods of Batschelet (1965) which involve the calculation of a mean vector using the sine and cosine of each individual angle. Two main parameters describe the mean vector: its angle, $\bar{\alpha}$, and the dispersion of the individual angles about it, r. The angular deviation, s, which is simply r measured in degrees, varies directly with dispersion and is therefore preferable (range, 0–81°). The hypothesis that the individual angles are uniformly distributed was tested using the V-test (Zar, 1984). Between-sex comparisons were made by testing the hypothesis that $\bar{\alpha}_\delta = \bar{\alpha}_\varphi$ using a parametric test statistic that is approximately distributed as Fisher's F (Batschelet, 1965).

We compared our results with previous studies of *A. maculatum* from other geographical areas. The data analysis for these studies follows that described for the Missouri animals. Because no investigator gave results in all formats, we performed several simple calculations in order to complete the comparison. When the raw data necessary to do these calculations were not available, either another test of significance was substituted or NA appears in place of the data.

Results

In 1985, we marked 645 salamanders, 510 males and 135 females. Because of the incomplete drift fence in 1985, the total number of immigrating salamanders in that year is unknown. In 1986, 38% of marked females and 30% of marked males returned. These propor-

TABLE 1. COMPARISON OF ORIENTATION PARAMETERS FOR ADULT *Ambystoma maculatum*. * indicates that χ^2 was the test statistic used, NA = data not available, n = sample size, $\bar{x}_r$ = expected mean # of drop cans if fence encounters are random, $\bar{x}_o$ = observed mean # of drop cans between fence encounters, % ± 1 = % of salamanders with a distance between fence encounters of one drop can or less, $\bar{\alpha}$ = angle of mean vector, s = angular deviation. Data for both sexes combined.

Study	n	# of drop cans				# of degrees		
		$\bar{x}_r$	$\bar{x}_o$	*P*	% ± 1	$\bar{\alpha}$	s	*P*
This study								
1985 IN–OUT	80	NA	3.9	<.001*	24	9°	47°	<.0005
1986 IN–OUT	67	6.5	3.1	<.001	45	1°	51°	<.0005
'85 IN–'86 IN	23	6.5	2.7	<.001	35	1°	53°	<.0005
Stenhouse, 1985								
'80 and '81 POOLED, IN–OUT	38	7.2	5.5	<.01	13	NA	NA	NA
'80 IN–'81 IN	12	7.2	3.8	<.001	NA	NA	NA	NA
Douglas and Monroe, 1981								
1974 IN–OUT	304	NA	NA	NA	26	358°	69°	<.01
Wilson, 1976								
'68 to '71 POOLED, IN–OUT	666	NA	NA	NA	22	NA	NA	NA
'68 to '71 POOLED, IN–IN	278	NA	NA	NA	20	NA	NA	NA
Shoop, 1968								
1964 IN–OUT	56	4.0	2.3	<.001	39	342°	54°	<.01
1965 IN–OUT	147	4.0	2.7	<.001	36	350°	62°	<.01
1966 IN–OUT	109	4.0	2.4	<.001	31	2°	57°	<.01

tions were not significantly different from that in which they were marked (2 × 2 contingency, $\chi^2 = 2.63$, $P > 0.05$). An additional 153 salamanders, 112 males and 41 females, were marked out of a total of 1351 (m–f sex ratio, 1.42:1) encountered in the outer drop cans during 1986.

The orientation parameters for the Missouri *A. maculatum* are given in Tables 1–2. Because previous investigators did not analyze their data by sex, our combined results are presented in Table 1. When measured in number of drop cans, all of the Missouri groups had means that were significantly different from the mean for random fence encounters (Tables 1–2). The results for the marked salamanders migrating in 1986 are shown graphically in Figure 1. Notice that a large proportion (45%) of the marked salamanders exited the pond within one drop can or less of their entry point and that no directional bias is apparent. Males and females did not differ significantly in their orientation

TABLE 2. BETWEEN-SEX COMPARISON OF ORIENTATION PARAMETERS FOR MISSOURI *Ambystoma maculatum* ($\bar{x}_r$ = 6.5). NA = data are not available, n = sample size, $\bar{x}_o$ = observed mean # of drop cans between fence encounters, % ± 1 = percentage of salamanders with a distance between fence encounters of one drop can or less, $\bar{\alpha}$ = angle of mean vector, s = angular deviation.

Group	n	# of drop cans			# of degrees		
		$\bar{x}_o$	*P*	% ± 1	$\bar{\alpha}$	s	*P*
1985 IN–OUT ♀	15	5.1	NA	7	44°	45°	<.005
1985 IN–OUT ♂	65	3.6	NA	28	1°	45°	<.0005
1986 IN–OUT ♀	16	3.8	<.02	44	354°	56°	<.0025
1986 IN–OUT ♂	51	3.0	<.001	45	3°	49°	<.0005
'85 and '86 POOLED, IN–OUT ♀	31	4.4	<.01	26	22°	55°	<.0005
'85 and '86 POOLED, IN–OUT ♂	116	3.3	<.001	35	2°	47°	<.0005
'85 IN–'86 IN ♀	11	2.6	<.001	18	339°	52°	<.005
'85 IN–'86 IN ♂	12	2.8	<.001	50	20°	51°	<.0025

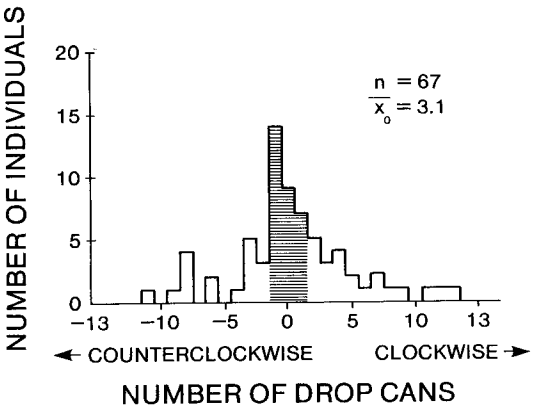

Fig. 1. Frequency distribution of the number of drop cans between entry and exit points for individually marked adult *Ambystoma maculatum* migrating in 1986. The shaded area represents the individuals that exited the pond within one drop can of their entry point. $\bar{x}_o$ was calculated without regard to sign.

ability either within years (1985, t = 1.65, $P > 0.1$; 1986, t = 0.53, $P > 0.5$), returning in consecutive years (t = 0.21, $P > 0.5$) or when the data for the 2 yr are pooled (t = 1.7, $P > 0.05$).

The re-analysis using degrees is also given in Tables 1–2. Again, all of the Missouri groups had r-values that were significantly different from the value for random behavior. In only one case was there a significant difference between the orientation ability of males and females as revealed by the parametric two sample test. Females, on the average in 1985, missed by more degrees ($F_{1,78} = 7.0$, $P < 0.05$). The other three comparisons between the sexes showed no significant differences.

The orientation parameters for the Missouri animals are in close agreement with those of the previous orientation studies. Of special interest are the parameters $\bar{\alpha}$, s, and % ± one drop can since $\bar{x}_o$ is measured relative to a particular drift fence. At least 20% of the animals in these five studies came within one drop can of exiting the breeding pond in the same spot in which they entered or entering at the same spot in two consecutive years.

Discussion

The patterns of entry-exit or entry-entry exhibited by the salamanders in this study show strong statistical directionality. In addition, previous studies from other geographical areas indicate that this may be a widespread phenomena in *A. maculatum*. There can be little doubt that *A. maculatum* is able to move between breeding and non-breeding habitats in a highly non-random fashion suggesting that these salamanders have some directional orientation ability. It should be noted that the drift fence/drop can method overestimates the distance between fence encounters (Shoop, 1965; Douglas and Monroe, 1981), so these data may be taken as minimum values.

That males arrive at the breeding pond earlier than females has been documented for many ambystomatid salamanders (Beneski et al., 1986). Possible explanations for this observation include: 1) differential rate or distance of travel; 2) differential response to environmental cues; and 3) differential orientation ability. Sexton et al. (1986), using group data, presented evidence in support of superior male orientation ability among years. Using individual data we conducted four intersex, pairwise comparisons with each orientation parameter. Seven of the eight comparisons showed no significant difference between the orientation ability of the sexes. The significant comparison, entry/exit in 1985, could be due in part to the small sample size of the females in that year (n = 15). It is important to note that these same data led to the opposite conclusion when analyzed using "number of drop cans." Thus, we have no strong evidence to support differing orientation ability between the sexes.

Skewed sex ratios in favor of males have also been reported with regularity, especially in *A. maculatum* (Peckham and Dineen, 1954; Whitford and Vinegar, 1966; Hillis, 1977; Sexton et al., 1986). Husting (1965) proposed that differential mortality between the sexes and/or a biennial mating pattern for females could be responsible. Both of these mechanisms should skew the sex ratio of a marked cohort, when followed through time, in favor of males. Our results do not support this, in fact, we show a slight but not significant bias in favor of females for the between-year recaptures. Delayed maturation of females (Wacasey, 1961) could account for the male-biased sex ratio.

Our results extend the documentation of migratory orientation to include *A. maculatum* from east-central Missouri. The similarity of these results to the previous studies is striking and lends support to adaptive explanations for orientation ability. Such behavior allows the salamanders to exploit an existing, suitable pond (Grubb, 1973; Gill, 1978; Stenhouse, 1985) and avoid excessive wandering. Late arrival at a

breeding pond could result in reduced reproductive success if early male immigrants are more likely to encounter receptive females (Arnold, 1976) or if early hatching of larvae provides an advantage in garnering the resources necessary for rapid growth and metamorphosis (Wilbur and Collins, 1973).

The question remains as to the mechanism(s) behind migratory orientation in *A. maculatum*. It is not clear whether familiar landmarks, simple compass orientation, or true navigation is being utilized (Schmidt-Koenig, 1965) or even which sensory modalities are being used. In studies of other salamanders, the use of celestial cues (Landreth and Ferguson, 1967; Taylor, 1972; Taylor and Adler, 1978), plane polarized light (Taylor and Adler, 1973), and magnetoreception (Phillips, 1986) has been identified. Future studies focused on this question should be encouraged as well as those dealing with sex ratio anomalies.

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