THE RESPONSE OF NAIVE BREEDING ADULTS OF THE SPOTTED SALAMANDER TO FISH

by

OWEN J. SEXTON*1, CHRISTOPHER A. PHILLIPS*1,2 and ERIC ROUTMAN2,4

(1 Department of Biology, Washington University, St. Louis, MO 63130; 2 Department of Anatomy, Washington University, St. Louis, MO 63130, U.S.A.)

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Summary

Several recent investigations have shown that some species of salamanders are capable of perceiving the presence of fish in breeding ponds through olfaction. It has been suggested that breeding adult salamanders may avoid depositing eggs in pools containing fish. We examined the hypothesis that breeding salamanders avoid fish ponds through olfactory cues in two field tests. In the first, naive spotted salamanders (Ambystoma maculatum) restrained within screened enclosures were submerged in either fish or fishless ponds. The number of eggs laid within each enclosure was counted at the conclusion of breeding. There was no difference in the number of eggs within and among four fishless and three fish ponds. In the fourth fish pond the fish harassed the salamanders and completely inhibited reproduction. We repeated the experiment using double screened enclosures in which the salamanders were free from direct fish attack but were still exposed to fish scent. There was no difference in egg production. The second test repeated the design of the first but provided the salamanders with the choice of remaining in the double screened enclosures or exiting the pond. The test animals from both the fish and fishless ponds responded similarly in their breeding activities. We conclude that naive spotted salamanders do not avoid fish ponds based solely on olfactory cues.

Introduction

It is well known that many species of amphibians which breed in aquatic situations are subject to intense predation by fish on adults, eggs, or larvae. The negative effects of this predator-prey relationship on ambystomatid salamanders have been well documented: restrictions in geographic distribution (Petranka, 1988; Semlitsch, 1988); changes in life history trajectories (Jackson & Semlitsch, 1993); and reduction in

* Current address: Illinois Natural History Survey, Champaign, IL 61820, U.S.A.
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growth rate and survival of larvae (Semlitsch, 1987; Figiel & Semlitsch, 1990). This last effect can lead to complete lack of larval recruitment (Ireland, 1989) and ultimately extirpation of salamander populations after invasion of predatory fish into a breeding pond (Burger, 1950; Sexton & Phillips, 1986). Other investigators have demonstrated that ambystomatid larvae can modify their behaviour in the presence of fish so as to reduce their probability of capture. These modifications usually take the form of alteration in diel pattern of foraging (Holomuzki, 1986; Stangel & Semlitsch, 1987) and refuge use (Sih et al., 1988; Figiel & Semlitsch, 1990; Huang & Sih, 1991; Sih et al., 1992) and may contribute to the negative effects outlined above.

Kats & Sih (1992) suggested that a selective advantage would accrue to those adult individuals which can reduce their reproductive activities in the presence of fish and demonstrated that stream pools without fish had significantly higher densities of Ambystoma barbouri eggs than did pools with fish. In accord with the selective advantage outlined above, they concluded that pools without fish are the preferred habitat for adult salamander oviposition. In our field work involving seining of hundreds of Midwestern ponds, we have also noticed a negative relationship between the presence of salamander eggs and larvae and the presence of fish.

Kats & Sih (1992) did not address the possible mechanisms through which adult salamanders detect fish. Adult salamanders could either detect waters containing fish and avoid them or abandon fish pools after being physically harassed by fish. It is known that salamander larvae are capable of detecting fish through chemical stimuli (Kats, 1988; Sih & Kats, 1991) and this suggests one possible method utilized by adult salamanders.

Our investigation examines the general hypothesis that adult spotted salamanders (Ambystoma maculatum) modify their reproductive behaviour in the presence of fish chemical cues. One specific hypothesis is that naive adults confined in screened enclosures which prevent physical contact with fish will deposit neither spermatophores nor eggs when placed in fish ponds. A second hypothesis is that adult salamanders offered a choice between depositing spermatophores or eggs or leaving the pond (i.e. unconfined salamanders) will choose the latter option when placed in screened enclosures in fish ponds.
Methods

The experiments were carried out during 1992 and 1993 at the Washington University Research Center in Western St. Louis County, MO. A series of descriptive and experimental investigations has been carried out on Ambystoma maculatum at this location (Seale & Boraas, 1974; Ward & Sexton, 1981; Sexton et al., 1986, 1990; Phillips, 1992; among others). Details of the study area are presented in those papers.

Naive adult salamanders were collected in drop-cans adjacent to drift fences positioned at right angles to known breeding migration pathways at Salamander Pond. We constructed this pond in 1965 and stocked it with A. maculatum eggs the following spring. The source of the eggs was Rankin Pond, a fishless pond 0.5 km south of Salamander Pond. Members of the Biology Department of this university have collected A. maculatum eggs at Rankin Pond since at least 1955. No fish were ever recorded during this period. Hence, the population from which experimental animals were withdrawn had never been exposed to fish from the mid-1930's until they were used in our experiments. We assumed that they were naive because of the lack of direct experience with fish. Salamanders were placed in one of several types of cages (see below) that were partially submerged in one of eight ponds. Details of the eight experimental ponds are given in Table 1. Salamanders exposed to fish were not returned to Salamander Pond at the conclusion of the experiments. All eggs retrieved from the cages were returned to the laboratory and held until they reached the neural crest stage.

The life history attributes of the six species of fish present in four of the ponds have been described by Plieger (1975). The generalized diets of the two minnows Pimephales notatus and Notemigonus crysoleucas include prey of the size of crustaceans and insects. Ward & Sexton (1981) demonstrated that P. notatus is not capable of extracting the eggs of Ambystoma maculatum from the thick egg membranes. The catfish Ictalurus melas has a diet similar to that of the minnows and all must be considered potential predators on recently hatched salamander larvae. The three other species, two sunfishes (Lepomis cyanellus and L. macrochirus) and the bass (Micropterus salmoides) are members of the highly predatory family Centrarchidae. Their diets include other fish, adult and larval amphibians, and crayfish.

Table 1. Descriptions of the study ponds. Fish density is the mean number of fish per standard seine haul

<table>
<thead>
<tr>
<th>Pond</th>
<th>Date built</th>
<th>Size</th>
<th>Maximum depth</th>
<th>Fish species</th>
<th>Fish density*</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. Twin</td>
<td>1965</td>
<td>45 m × 25 m</td>
<td>2.5 m</td>
<td>Pimephales promelas</td>
<td>76</td>
</tr>
<tr>
<td>Spring</td>
<td>1970</td>
<td>12 m diam.</td>
<td>1.5 m</td>
<td>Pimephales promelas</td>
<td>522</td>
</tr>
<tr>
<td>Pumphouse</td>
<td>1979</td>
<td>5 m × 3 m</td>
<td>1.5 m</td>
<td>Ictalurus melas</td>
<td>26</td>
</tr>
<tr>
<td>Salamander</td>
<td>1965</td>
<td>45 m × 5 m</td>
<td>1 m</td>
<td>Notemigonus crysoleucas</td>
<td>91</td>
</tr>
<tr>
<td>New</td>
<td>1968</td>
<td>15 m diam.</td>
<td>1 m</td>
<td>Lepomis cyanellus &amp; L. macrochirus</td>
<td>170</td>
</tr>
<tr>
<td>Railroad</td>
<td>1970</td>
<td>42 m × 36 m</td>
<td>1 m</td>
<td>Micropterus salmoides</td>
<td>2</td>
</tr>
</tbody>
</table>

* mean number per seine haul.
Hypothesis 1.

We utilized two experimental designs to test Hypothesis 1, that naive adults confined in wire cages will deposit neither spermatophores nor eggs when placed in fish ponds. For experiment 1A, which was conducted in 1992, the cages were fabricated from a single layer of 6 mm mesh hardware cloth. Cage height was 46 cm and diameter was 40 cm. Each cage was supported from a vertical pole with the upper 1/4 of the cage protruding above the water surface. We placed leaves and twigs within the cylinder to facilitate deposition of spermatophores and eggs. Four adult males and two adult females were placed within each cage the morning after they attempted to enter Salamander Pond. We selected this ratio of sexes because it approximates the ratio in Salamander Pond during the breeding migration (Sexton et al., 1986). We placed four or five cages in each of four fish and four fish-free ponds (Table 2). We examined the cages daily for the presence of spermatophores and egg masses. When eggs masses were no longer being deposited, we counted the number of eggs deposited within each cage and measured the snout to vent length SVL of each female to the nearest mm.

For experiment 1B, which was conducted in 1992 and 1993, the cages were modified after the first completed run of experiment 1A for reasons that will be apparent in the Results section. The type 1A cylinders were surrounded by larger cylinders constructed of the same mesh. These double-walled cylinders prevented any physical contact between fish and salamanders, yet for both types 1A and 1B, water dispersed scents could penetrate the cage interior. We placed leaves, twigs, and four male and two female salamanders within the inner cylinder of each apparatus. Four or five of these modified cages were placed in Salamander Pond and Railroad Pond (Table 3). We examined the cages daily for the presence of spermatophores and egg masses. When eggs masses were no longer being deposited, we counted the number of eggs deposited within each cage in 1992 and the number of egg masses in 1993. In 1992, we also measured the SVL of each female to the nearest mm.

Hypothesis 2.

The concern that egg and spermatophore deposition might simply be a reaction to confinement—see Discussion—prompted a third experimental design. Experiment 2 tested the hypothesis that naive salamanders offered a choice between depositing eggs or spermatophores or leaving the pond unconfined salamanders will choose the latter when placed in fish ponds. The test apparatus for experiment 2, which was run in 1993, consisted of two concentrically arranged cylinders. 2 m long, constructed of window screen 0.3 mm mesh. The diameter of the inner cylinder was 18 cm and that of the outer cylinder was 30 cm. One end of the apparatus was submerged in shallow water near the edge of the pond. Separate plastic bags covered both the inner and outer cylinders of this end. Pond water could flow through the submerged end but fish could not contact the salamanders. The other end of the cylinder rested on the shore of the pond. The land end of the inner cylinder had a flexible plastic tube that dropped into a bucket so that animals choosing to leave the cages were captured. We placed leaves, sticks, and 4 male and 2 female salamanders in the submerged end of the inner cylinder. Five of these modified cages were placed in Pumphouse Pond and five in Railroad Pond (Table 4). Pumphouse Pond was used as a fishless pond instead of Salamander Pond in order to avoid the confounding effect of home pond that Salamander Pond conferred. Each day we counted the number of salamanders that had fallen from the inner tube into the land bucket. At the conclusion of the experiment we removed the apparatus and counted the number of egg masses within its submerged portion.

For all experiments, we tested the specific null hypothesis that there would be no significant difference in the reproductive behavior of the salamanders in the fish ponds compared to the fishless ponds. The unit of comparison was the number of eggs experi-
ments 1A and 1B, 1992; or egg masses (experiments 1B, 1993 and 2, divided by the number of females in a cage (because some females escaped during the first night of confinement in Experiment 1). This is referred to as the normalized number of eggs (or egg masses) per cage. The effects of fish treatment were tested using Analysis of Variance (ANOVA) with SVL as a covariate in experiments 1A and 1B, 1992.

Results

Experiment 1A.

This experiment lasted 13 days. Spermatophores were deposited in all cylinders during the first or second nights after the start of the experiment. Oviposition took place on the third and fourth nights and occurred in all cages in all ponds except Railroad Pond. The salamanders in the cages from Railroad Pond were mutilated, presumably the result of being bitten and pulled against the screen mesh by fish. After removing the variation explained by SVL, there was no significant difference between fish treatment levels in the normalized number of eggs per cage (Table 2) if the Railroad Pond cages were excluded (ANOVA, $F = 2.369$, $df = 5$, $p = 0.067$).

Experiment 1B.

This experiment lasted 11 days in 1992 and 12 days in 1993. Spermatophores were deposited in all cylinders during the first or second night after the start of the experiment in both 1992 and 1993. Oviposition took place on the third and fourth nights and occurred in all cages in both

<table>
<thead>
<tr>
<th>Table 2. Summary for experimental series 1A</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fishless Pond</td>
</tr>
<tr>
<td>----------------</td>
</tr>
<tr>
<td>East Twin 4</td>
</tr>
<tr>
<td>± 0.19</td>
</tr>
<tr>
<td>Spring 5</td>
</tr>
<tr>
<td>± 0.18</td>
</tr>
<tr>
<td>Pump House 5</td>
</tr>
<tr>
<td>± 0.19</td>
</tr>
<tr>
<td>Salamander 5</td>
</tr>
<tr>
<td>± 0.13</td>
</tr>
</tbody>
</table>

* = number of cages used in each pond. SVL = mean snout-vent length of females in cm ± 1 S.E. N.A. = data not available. * = Number of eggs divided by number of females in a cage averaged over cages in a pond ± 1 S.E.
1992

<table>
<thead>
<tr>
<th>Fishless Pond</th>
<th>SVL</th>
<th># Eggs*</th>
<th>Fish Pond</th>
<th>SVL</th>
<th># Eggs*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salamander (4)</td>
<td>8.99</td>
<td>± 0.37</td>
<td>Railroad (4)</td>
<td>8.87</td>
<td>± 2.23</td>
</tr>
<tr>
<td></td>
<td>151.25</td>
<td>± 8.92</td>
<td></td>
<td>140.5</td>
<td>± 26.31</td>
</tr>
</tbody>
</table>

1993

<table>
<thead>
<tr>
<th>Fishless Pond</th>
<th>SVL</th>
<th># Egg Masses*</th>
<th>Fish Pond</th>
<th>SVL</th>
<th># Egg Masses*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salamander (4)</td>
<td>NA</td>
<td>1.8 ± 0.200</td>
<td>Railroad (4)</td>
<td>NA</td>
<td>1.7 ± 0.200</td>
</tr>
</tbody>
</table>

( ) = Number of cages used in each pond. SVL = mean snout-vent length of females in cm ± 1 S.E. N.A. = data not available. * = Number of eggs or egg masses divided by number of females in a cage averaged over cages in a pond ± 1 S.E.

ponds in both years (Tables 2 and 3). For the 1992 experiment, there was no significant difference between the fish treatments in the normalized number of eggs per cage (ANOVA, F = 0.203, df = 1, p = 0.671) and there was no significant effect of SVL on the normalized number of eggs per cage (ANOVA, F = 0.513, df = 1, p = 0.506). For the 1993 experiment, there was no significant difference between the fish treatments in the normalized number of egg masses per cage (ANOVA, F = 0.125, df = 1, p = 0.733). SVL was not measured in 1993.

Experiment 2.

This experiment lasted 5 days. Spermatothores were deposited in all cylinders by the second night of the experiment. Oviposition occurred in three of the fish treatment cages and three of the fishless treatment cages. The normalized number of egg masses per cage was the same in the two treatments (Table 4). SVL was not measured in experiment 2. In the fishless pond 13 males and 4 females left the water end of the apparatus and were captured in the terrestrial end. For the fish pond the numbers were 16 and 4 for the same 24 hr period.

Discussion

Hypothesis 1 stated that there would be no difference between the breeding behaviour of naive spotted salamanders confined in single-layered
mesh enclosures in fish and fishless ponds. The original intent of the experiments testing this hypothesis was to measure the effectiveness of water-borne fish scent in reducing reproductive activity. An inadvertent design flaw of experiment 1A confounded this intent because direct physical harassment of salamanders by fish occurred in Railroad Pond. We believe that this harassment contributed significantly to the lack of egg deposition in Railroad Pond and deflated the overall level of egg deposition in fish ponds. If one compares the four fishless ponds to the other three fish ponds, there is no significant difference in the number of eggs deposited. This explanation is supported by the results of Experiment 1B in which physical harassment is precluded by the double walled cages. Under these conditions, Hypothesis 1 was not rejected; there was no significant difference in the number of eggs or egg masses deposited in fish versus fishless ponds.

It could be argued that animals confined to an enclosure might be physiologically induced to deposit spermatophores and eggs simply as a response to stress. Hypothesis 2 was designed to examine this possibility. Expectations were that, if salamanders were sensitive to water-borne fish odor, salamanders in Railroad Pond should leave the pond whereas those at Pump House Pond should remain in the pond and breed. Our data indicate that the two sets of unconfined salamanders behaved similarly.

Even though experiments 1B and 2 were not replicated, the fact that all experiments concur in their basic results lends credibility to our argument. However, even if minor quantitative differences in the number of eggs laid had been detected between the treatments this would not have diminished the argument that these salamanders are not altering their behavior in the presence of fish. It is more important that eggs were laid

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**Table 4. Summary for experimental series 2**

<table>
<thead>
<tr>
<th>Fishless Pond</th>
<th># Egg Masses</th>
<th>Fish Pond</th>
<th># Egg Masses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pump House (4)</td>
<td>0.6 ± 0.292</td>
<td>Railroad (4)</td>
<td>0.6 ± 0.292</td>
</tr>
</tbody>
</table>

# emigrants: 13 ♂, 4 ♀  
# emigrants: 16 ♂, 4 ♀

(1) Number of cages used in each pond. Number of emigrants are from first 24 hrs of experiment. * = Number of egg masses divided by number of females in a cage averaged over cages in a pond ± 1 S.E.
in both treatments. It is also significant to note that the timing of oviposition did not differ between treatments within any of the experiments. We know from our own observations that spotted salamanders, when held in captivity, can postpone laying viable eggs for several days. Therefore, it should have been possible for our confined salamanders to delay oviposition as a response to the presence of fish.

Overall, our study does not support the hypothesis that reproductive activities of naive adult *A. maculatum* are inhibited by the presence of fish scent, either a generic "fish" smell or a specific one from known predators. Instead, we suggest that physical harassment is the proximate mechanism responsible for any avoidance of fish ponds by adult salamanders. Since our work dealt with naive salamanders, we do not eliminate the possibility that adult salamanders that have survived harassment may learn to associate harassment with fish odor and thereby avoid fish ponds later in life. In fact, this may be the explanation for the observations made by Kats & Shih (1992) in their study of *A. barbouri* oviposition in fish and fishless stream pools. Perhaps contact with fish either as larvae or as adults could modify adult breeding behaviour. However, this is in contrast to Ireland's (1989) study of survivorship of *A. maculatum* larvae in a fish and a fishless pond in Virginia. The former pond had contained fish for approximately 30 years. During the course of Ireland's study, no transformed larvae left the fish pond but adult salamanders continued to breed there in approximately constant numbers. There are two explanations to account for this observation: (1) long-lived adults do not select breeding ponds on the basis of successful recruitment; (2) some members of a "floating population" (i.e. adults not attached to a particular pond, sensu Sexton & Phillips, 1986) utilized this pond. Ireland concluded that female spotted salamanders do not select breeding ponds on the basis of successful recruitment. Our results support this hypothesis.

References


RESPONSE OF NAÏVE SALAMANDERS TO FISH


