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**Sex Differences and Seasonal Trade-Offs in Response to Injured
and Non-Injured Conspecifics in Red-Spotted Newts,
*Notophthalmus viridescens***

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Sex differences and seasonal trade-offs in response to injured and non-injured conspecifics in red-spotted newts, *Notophthalmus viridescens*

Abstract Injured prey often release alarm chemicals that induce antipredator behaviors in conspecifics. Injured or killed prey most likely release a wide array of chemicals in addition to alarm substances, such as sexual pheromones, which could enhance or compromise antipredator responses. Thus, damage-release cues provide an excellent opportunity to examine the influence of seasonally fluctuating sexual pheromones on antipredator behaviors. We used a series of laboratory and field experiments and meta-analysis to examine seasonal changes and sex differences in the response of red-spotted newts, *Notophthalmus viridescens*, to the odor of non-injured conspecifics and conspecific tissue extracts, the latter of which presumably contain pheromones of non-injured conspecifics combined with alarm chemicals signaling predation. During the peak of the breeding season, males were attracted to females and multiple males, but did not avoid tissue extracts from either sex. As the breeding season waned, male attraction to females and males decreased, while avoidance of alarm extracts from both sexes concurrently increased. In contrast to male behavior, females were indifferent to both sexes during the breeding season, and showed significant avoidance only of female extract. As the breeding season progressed, females displayed no change in response to treatments. Male and female responses to female rinse and extract differed significantly, but their response to male treatments did not. During the non-breeding season, both males and females were indiffer-

ent to the odor of conspecifics and avoided conspecific tissue extracts, with the magnitude of male avoidance greater than that of female avoidance, suggesting sex differences in response to alarm cues in both the breeding and non-breeding seasons. In general, both male and female response to conspecific odor and tissue extracts covaried positively, suggesting that social pheromones can be detected within conspecific macerates and compromise alarm-chemical avoidance. Many of the sex differences in both seasons are likely explained by selection pressures imposed on males to intensely mate search during the breeding season, suggesting that the mating system of newts directly influences predation threat during reproductive activity and may have significant indirect consequences on risk during the non-breeding season.

Keywords Antipredator behavior · Alarm chemical · Seasonal variation · Conflicting chemical cues · Salamanders

Introduction

Most animals face predation risk at some point in their lives, underscoring the importance of thoroughly examining the dynamics of predator-prey interactions for evolutionary trade-offs. Appropriate antipredator responses are crucial to prey since, in most cases, survival for prey is of greater fitness consequence than a meal for a predator. In addition, prey must also acquire food and mates, and in many cases, these activities can compromise their antipredator responses (Lima and Dill 1990). For example, attraction to food may result in prey being less responsive to predator cues (e.g., Hazlett 2000; Toomba et al. 2001). Conspecific interactions among prey provide potentially more complex and interesting effects on antipredator behavior than responses to food because conspecifics can be attractive or repulsive at different times (e.g., Madison and McShea 1987), and are thus capable of compromising or reinforcing anti-

predator behaviors. Responses to conspecifics perhaps vary most profoundly in prey that have distinct breeding and non-breeding seasons, with the greatest predation threat often occurring during reproductive activities (Lima and Dill 1990).

During the breeding season, at least one gender is usually attracted to the opposite sex, often resulting in pronounced sex differences in behavior and predation. For example, mate search can increase encounters with predators (Sakaluk and Belwood 1984; Gwynne 1987; Moore 1987), and displays and calls that attract mates can increase conspicuousness (Ryan 1985; Endler 1987; Trail 1987), which can differentially affect male and female predation risk depending on which sex exhibits the conspicuous reproductive activity. Sex differences during the breeding season can become further pronounced if there is a substantial discrepancy between genders in the variance of mating success. During the breeding season, the sex with more variable reproductive success often benefits from risky behavior because the potential pay-offs can be greater (Magurran and Garcia 2000).

Reproductive activities have obvious costs during the breeding season, but less obvious, and perhaps just as important, are the indirect costs of prey mating systems during the non-breeding season (Magurran and Garcia 2000). Sexually selected behaviors may persist, to varying degrees, into non-breeding periods, and these behaviors may be correlated with predation risk, potentially posing constant conflicting sexual and natural selection pressures (Kats and Dill 1998; Magurran and Garcia 2000). For example, risky behaviors of some male fish (due to more variable mating success) are purported to be frequently and non-adaptively expressed even in the absence of mates, increasing their predation risk during all times of the year (Magurran and Garcia 2000). The sex primarily responsible for mate search may have been selected to have more acute senses to locate mates (e.g., Dawley 1998), and this extra acuity could secondarily facilitate predator detection (Kats and Dill 1998), resulting in sex differences in predator avoidance and predation across seasons.

Despite the complex interplay between gender differences, social behaviors, and predator avoidance across seasons, few studies have investigated the relationship between seasonal change in response to conspecific and predator cues for male and female prey. Although resources and predators are often detected using chemoreception (Dodson et al. 1994; Kats and Dill 1998), few studies have investigated prey responses to complex chemical environments (Chivers and Smith 1998; but see Petranks 1989; Hazlett 1996, 1999, 2000; Toomba et al. 2001). Even fewer studies have explored social chemicals mixing with those released during predation (but see Kerby and Kats 1998; Rohr and Madison 2001b), and none have considered the sex and seasonal response dynamics of conspecific prey to this commonly occurring mixture. These gaps in the literature on chemically mediated predator-prey interactions prompted us to assess

whether seasonal change in the response of red-spotted newts, *Notophthalmus viridescens*, to rinses from non-injured conspecifics was correlated with their response to conspecific tissue extracts, which contain alarm chemicals (Marvin and Hutchison 1995).

Much is known about the response of red-spotted newts to chemicals from injured and non-injured conspecifics. During the non-breeding season, skin extracts from injured *N. viridescens* induce avoidance in conspecifics in both laboratory and field tests (Marvin and Hutchison 1995; Woody and Mathis 1997). During the breeding season, male *N. viridescens* are attracted to the odor of females and multiple males (Dawley 1984; Park and Propper 2001), females are indifferent to the odor of both sexes (Dawley 1984), and female odor compromises male alarm-chemical avoidance (Rohr and Madison 2001b). However, four major questions regarding this system remain: (1) do males and females respond differently to conspecific alarm chemicals; (2) do responses to conspecific alarm chemicals change through the breeding season; (3) do responses to conspecific odors and alarm chemicals differ between the breeding and non-breeding season; and (4) do sexual pheromones released with alarm substances compromise or enhance antipredator behaviors of conspecifics?

We used a series of laboratory and field experiments to quantify the change in response of male and female *N. viridescens* to odor of injured and non-injured conspecifics of both sexes during the breeding and non-breeding season. During the peak of the breeding season, we expected male attraction to female and multiple male odors to offset alarm-chemical avoidance. As the breeding season waned, we predicted increased avoidance of alarm substances because attraction to sex pheromones was expected to decline. No hypotheses were made regarding female response to extracts or conspecifics during the breeding season; however, male and female response to both conspecifics and extracts was expected to differ significantly during the breeding season because of different sexual motivations. During the non-breeding season, we predicted that males and females would avoid macerated tissue extracts from both sexes because sex pheromones would no longer be produced, resulting in a significant change in behavior for males across seasons. Finally, since newts use olfaction to detect mates (Dawley 1984; Cogălniceanu 1994), and since males are the primary mate searcher (Verrell 1982), males were expected to be more attracted to conspecifics, especially females, resulting in the largest sex difference in response to rinses and extracts during the breeding season. During the non-breeding season and toward the end of the breeding season (when conspecific odors were not expected to conflict with newt antipredator response), males were expected to exhibit greater avoidance of alarm chemicals than females because intense selection pressures on males to locate mates during the breeding season were expected to have conferred males with greater chemical sensing acuity during non-reproductive periods.

Methods

Laboratory experiments

We collected adult newts for the two breeding-season experiments in late March and late April 2001 from a private pond in Chenango County, New York, United States, and immediately placed individuals into labeled 0.47-l transparent, plastic containers filled with aged tap water. Plastic holding containers were maintained in a controlled environmental chamber at 18°C on a 14:10 h light:dark cycle, and newts were fed a liberal diet of chopped earthworms and freeze-dried bloodworms every week. Individuals were returned to their assigned container after each trial, facilitating individual identification throughout the experiment.

Adults for the non-breeding-season experiment were collected at the end of the breeding season in late May 2000 (from same population) because adults are difficult to catch during the non-breeding periods. Non-breeding season trials occurred approximately 3 months after collection, from late August to early September 2000, to ensure that reproductive motivation had subsided. During the 3 months prior to the non-breeding-season experiment, no more than 15 same-sex newts were held in 38-l aquaria containing aged tap water and simulated vegetation. Three days prior to trial commencement, newts were placed into the same water-filled, plastic containers used to hold newts for breeding-season trials. Newts for the non-breeding-season experiment were maintained in the same environmental chamber and under the same conditions as newts used in the breeding-season experiments.

Five treatments were used in laboratory experiments: rinses from uninjured male and female newts, macerated tissue extracts from male and female newts, and control water. Rinses were acquired from two males and two females placed into separate bowls containing 500 ml distilled water for 48 h. This water was then filtered through glass wool and 18-ml aliquots were immediately frozen. Extracts were obtained by sacrificing the same males and females used for rinses, macerating each pair (entire animal) in 350 ml distilled water, filtering through glass wool, and immediately freezing 18-ml aliquots. Males and females were in breeding condition (black pigment on hind legs for males and on cloaca of females, Petranka 1998) for breeding-season trials only. Aliquots of distilled water were also filtered and frozen to be used as control treatment. Disposable surgical gloves were used for all stages of treatment and trial preparation to minimize chemical contamination of treatments.

All behavioral tests of preference were conducted using a petri-dish design. Two semi-circles of filter paper were placed into each of 50 petri dishes (15 cm diameter × 1.5 cm height), and separated by a 3-mm gap that minimized mixture of fluids between halves. All treatment solutions were thawed just before trials. One semi-circle in each dish received 1.5 ml control water, and the other semi-circle received 1.5 ml male newt extract (MNE), female newt extract (FNE), male rinse, female rinse, or control water. Air bubbles under each filter paper were gently rolled to the outer edge so that the topography on both semi-circles was similar.

Petri dishes were randomly positioned and oriented (control side on right or left) on a grid placed on the floor of a test room, which was free of extraneous light sources. Individuals were removed from their holding cups, blotted free of excess water, and then placed in the center of their assigned test dish. Overhead videotaping began just before individuals were distributed, and opaque paper collars were placed around each dish during distribution to visually separate newts. Once all newts were in test dishes, lights were turned off and infrared videotaping commenced for 100 min. All tests began between 1400 and 1530 hours; 1 day was provided between trials to allow newts to re-hydrate, and individuals were exposed to each treatment once in random order. This resulted in a sample size of 25 for each sex exposed to each treatment for the April and May breeding-season trials, and a total sample size of 50 for each sex exposed to each treatment for both the breeding season (April+May trials) and non-breeding-season experiments.

We recorded the side that each newt occupied every 2 min for the last 80 min of video, which provided an initial 20-min acclimation period for each newt. If newts straddled the gap between semi-circles, the location of their snout was used as the criterion of choice (Marvin and Hutchison 1995). Most newts chose a side of the dish and then remained immobile for much of the remainder of the trial. This resulted in bimodally distributed data, which lends itself to binomial analysis. We were interested in the interactions between treatment, sex, and time of year, and unlike most non-parametric tests, binomial regression allows for the examination of interaction terms. Thus, treatment "avoidance" was scored if ≥ 21 of 41 observations were on the control side, and binomial regression with a log-log canonical link in Statistica's (Statsoft, 1998 version 5.5A) Generalized Linear Model (GZLM) was used to test the full factorial model, with factors sex (male, female), season (breeding, non-breeding), and treatment (male and female extracts and rinses). We tested for significant effects using the Wald statistic (analogous to least-squares estimates), rather than likelihood ratios, due to its ease of computation (Dobson 1990). Chi-square goodness-of-fit tests were used to assess preference for treatment or control substrates. To regain the statistical power sacrificed by using binomial analyses (not considering the number of observations on each side of the dish), we used mean locations in a Mann-Whitney *U*-test to compare male and female responses to rinses and extracts in the non-breeding season (Siegel and Castellan 1988).

Field experiments

We conducted an experiment at the fishless Nuthatch Hollow pond during late May and early June of 2000 to examine newt response during a "snap shot" of the breeding season. All field experiments used a minnow trap design described by Rohr and Madison (2001b). In brief, we placed a frozen sponge (1 × 2.5 × 4 cm) containing 5 ml of either MNE, FNE, or control water, or a frozen control sponge plus 2 females (2F), 2 males (2M), or 4 males (4M) in each of 24 traps spaced a minimum of 5 m apart and 1 m from shore. Similar concentrations of MNE and FNE as those used in the laboratory experiment were made from two male or two female conspecifics in breeding condition. Each trap randomly received each treatment until all traps had received only each treatment once. This procedure was then repeated so that each trap received each treatment twice over a 12-day period. This newt population was sufficiently dense that recapturing the same newt in the same treatment (pseudoreplication) was presumed to be unlikely. Traps were set between 1230 and 1400 hours, and the numbers of captured male and female newts were counted 2 h later.

We used GZLM for a Poisson distribution with a log canonical link to compare treatments (McCullagh and Nelder 1989). However, since response was not examined across season, only Bonferroni adjusted linear contrasts were made (family $\alpha=0.1$). At this portion of the breeding season, avoidance of injured conspecific males or females (MNE or FNE) was only expected to occur if there was no attraction to the odors of the same number and sex of non-injured conspecifics. Similar field experiments were attempted during the non-breeding season, but capture rates were too low (3% of traps capturing newts) to justify further sacrifice of newts to reach sample sizes providing adequate statistical power.

In an attempt to field validate our laboratory findings examining newt response through the breeding season, we performed meta-analysis on data collected from multiple experiments conducted during 2000 and 2001 (between February and June) at Binghamton University's Nature Preserve and Nuthatch Hollow (Broome County, N.Y., USA) populations. A similar methodology used for the Nuthatch Hollow snap-shot experiment was used for all experiments incorporated into the meta-analyses. Traps were distributed a minimum of 5 m apart and approximately 1 m from the shore of the two ponds. Depending on the experiment, traps contained 5 ml MNE, FNE (similar to laboratory concentrations), or control water on frozen cellulose sponges, or one live female conspecific, three or four live male conspecifics, or nothing. The

number of captured male and female newts and fish were counted 50, 100, 120, or 150 min after traps were set. Bluegill sunfish, *Lepomis machrochirus*, the predominant fish captured in Binghamton University traps, did not affect newt captures in minnow traps in a previous laboratory experiment (Rohr and Madison 2001a), and therefore are not considered in this paper.

In most experiments, trap locations received each treatment once, and capture rates between treatments were compared within each location to control for microhabitat differences. For meta-analysis, only trap locations that captured newts in either control or treatment traps were included. Therefore, if only the treatment or control trap caught a newt at a given location, the treatment or control trap at that location that did not catch newts was included in the analyses, but if neither treatment nor control traps at a location caught newts, they were both excluded. This procedure controlled for known activity declines across the breeding season that influence the percentage of traps that capture newts (J.R. Rohr and D.M. Madison, unpublished data).

The breeding season was divided into 2-, 4-, or 6-week periods depending on whether the division produced sample sizes deemed large enough for analysis. To estimate male response, trap locations that captured females were excluded because males at these sites are strongly attracted to female odor during the breeding season (Rohr and Madison 2001b), which could offset or augment treatment control differences. Sample sizes were large enough to analyze seasonal change in male response to each treatment at the Binghamton University population, but were only large enough to analyze seasonal change in response to MNE at Nuthatch Hollow. For female response, all trap locations that caught females at both populations were combined because female captures, and thus sample sizes, were minimal. Too few females were captured in FNE and FNE control traps to warrant statistical analysis. Experiments containing MNE and MNE control traps had the greatest sample size and overlap in portions of the two field seasons, and were thus used to test for year differences. These data revealed no significant main effects or interactions containing year for either sex; to preserve degrees of freedom, we ran all subsequent tests excluding year.

For male captures, we used Poisson regression with the recommended log canonical link (McCullagh and Nelder 1989) and trial duration as a covariate to test for treatment response change through the breeding season. For all treatments, this model resulted in a good fit, with deviance scores very close to 1 (1.1–1.3; McCullagh and Nelder 1989). ANOVA rather than ANCOVA was used to test effects of conspecific male and FNE presence because trial duration did not vary (120 min). Female captures did not fit a Poisson distribution, so Wilcoxon matched pairs (*T*) tests were used to compare captures in control and treatment traps, and Kruskal-Wallis (*H*) tests were used to compare treatment effects during portions of the breeding season.

Results

Laboratory experiments

As predicted, fewer newts avoided treatments during the breeding than non-breeding season; there was a significant main effect of treatment, and male attraction to treatments decreased from the breeding to non-breeding season significantly more so than did female attraction, producing a sex by season interaction (Table 1, Fig. 1). Males were significantly attracted to male and female rinse only during the breeding season, while females were indifferent to conspecific rinses during both seasons. The non-significant season by treatment interaction indicates that responses to rinses and extracts changed similarly over time; as attraction to conspecifics decreased, attraction to extracts also declined (Table 1, Fig. 1).

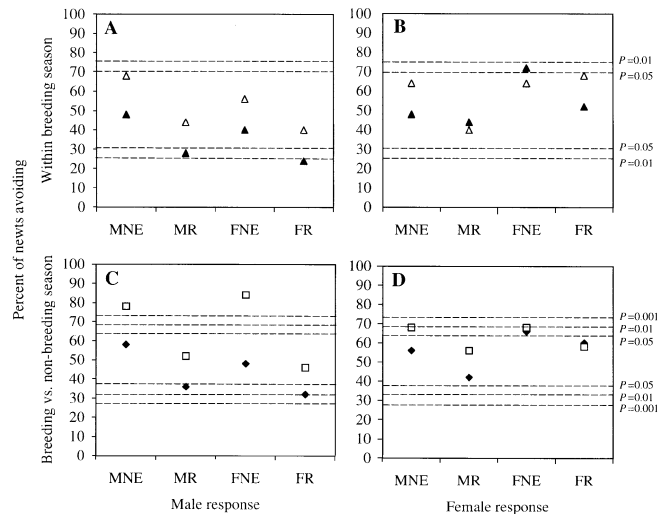


Fig. 1A–D Percent of male and female newts avoiding macerate male (MNE) and female newt extracts (FNE) and male (MR) and female rinses (FR) through the breeding season (4/6/01–4/14/01=unfilled triangles, 5/1/01–5/9/01=filled triangles, $N=25$) and between the breeding (unfilled squares, $N=50$) and non-breeding (filled diamonds, $N=50$) seasons in laboratory trials. Probability thresholds for chi-square goodness-of-fit tests are also shown

Table 1 Results of binomial regression for effect of sex (male, female), season (breeding, non-breeding), and treatment (male and female extracts and rinses) on newt treatment avoidance

Factor	<i>df</i>	Wald	<i>P</i>
Sex	1, 784	2.93	0.09
Season	1, 784	17.48	<0.001
Treatment	3, 784	29.80	<0.001
Sex×season	1, 784	4.83	0.03
Sex×treatment	3, 784	7.23	0.06
Season×treatment	3, 784	1.53	0.68
Sex×season×treatment	3, 784	3.28	0.35

At the estimated peak of the breeding season in Binghamton University's Nature Preserve (from 6 to 14 April 2001, J.R. Rohr, unpublished data), males were significantly attracted to male and female rinses, but did not avoid their extracts, while females appeared indifferent to all treatments except for avoiding FNE (Fig. 1A, B). As the breeding season waned from 1 to 9 May 2001, males were no longer significantly attracted to conspecifics, and the number of males avoiding conspecific extracts increased (Fig. 1A). At the same time, females showed no significant avoidance or attraction to extracts or rinses (Fig. 1B). Overall during the breeding season, males showed significant attraction to rinses, but no avoidance of extracts, and females avoided FNE, but were indifferent to all other treatments (Fig. 1C, D). Male and female responses to male extract ($N=50$, $\chi^2=0.04$, $P=0.84$) and rinse ($N=50$, $\chi^2=0.38$, $P=0.54$) did not differ significantly during the breeding season. However, males were significantly more attracted to female extract ($N=50$, $\chi^2=4.11$,

Table 2 Two-way ANOVA testing effects of breeding-season portion and MNE (male newt extract) and conspecific female presence on male *Notophthalmus viridescens* captures controlling for trial duration. ANOVA rather than ANCOVA was used to test

effects of conspecific male and FNE (female newt extract) presence because trial duration did not vary. The Generalized Linear Model for a Poisson distribution was used for analyses. See Fig. 1 for breeding-season portions and sample sizes

Factor	Treatments											
	MNE			3-4 males			FNE			Female		
	df	Wald	P	df	Wald	P	df	Wald	P	df	Wald	P
Trial duration	1	16.71	<0.001	—	—	—	—	—	—	1	9.50	0.002
Breeding season portion	4	30.17	<0.001	2	3.98	0.14	2	9.18	0.01	2	3.26	0.20
Treatment presence	1	4.16	0.04	1	13.08	<0.001	1	2.94	0.09	1	12.90	<0.001
Breeding season portion×treatment	4	21.36	<0.001	2	2.86	0.24	2	3.11	0.21	2	8.72	0.01

$P=0.04$) and rinse ($N=50$, $\chi^2=7.89$, $P=0.005$; Fig. 1) than were females.

During the non-breeding season, both males and females were indifferent to conspecific rinses but avoided extracts (Fig. 1C, D). Male and female response to rinses did not differ significantly ($N=100$, $U=4618.0$, $P=0.35$), but the magnitude of male extract avoidance was greater than that of female extract avoidance ($N=100$, $U=4179.0$, $P=0.04$).

Field experiments

During the Nuthatch Hollow snap-shot experiment (late May to early June), when males were attracted to a specific number of conspecifics, they did not avoid alarm chemicals attained from the same number of conspecifics, as predicted. Males were attracted to two females (Wald_{1, 94}=9.64, $P=0.002$), but did not avoid extract from two macerated females (Wald_{1, 94}=0.67, $P=0.41$; Fig. 2). Males were not significantly attracted to two males (Wald_{1, 94}=0.01, $P=0.91$), but did avoid extract from two males (Wald_{1, 94}=6.67, $P=0.009$). Four males caught more newts than two males although the difference was not significant following Bonferroni adjustments (Wald_{1, 94}=4.84, $P=0.03$). Finally, two females induced greater male attraction than two males (Wald_{1, 94}=10.32, $P=0.001$), but attraction to two females and four males did not significantly differ (Wald_{1, 94}=1.07, $P=0.30$; Fig. 2). Too few females were captured in each treatment (C=1, 2F=1, 2FNE=2, 4M=1, 2M=3, 2MNE=0) to justify analysis.

Meta-analyses revealed that, during the breeding season, females did not significantly avoid MNE ($N=62$, $T=745.5$, $P=0.21$, Fig. 3E), even though traps containing MNE caught fewer females than control traps during every portion of the breeding season. There was also no significant difference in capture rates between portions of the breeding season ($H=0.58$, $df=2$, 124, $P=0.76$). Furthermore, females were neither attracted to, nor avoided, male ($N=23$, $T=105.0$, $P=0.72$) or female conspecifics ($N=42$, $T=400.5$, $P=0.70$), and neither response changed significantly through the breeding season

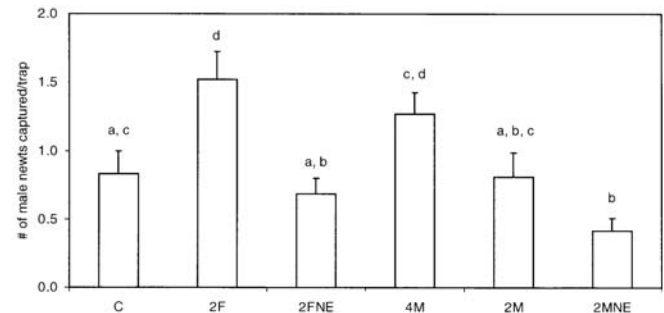


Fig. 2 Capture rates of *Notophthalmus viridescens* in the presence and absence of conspecifics and conspecific tissue extracts at Nuthatch Hollow during late May and early June of the 2000 breeding season. Traps contained 2 females (2F), 2 males (2M), 4 males (4M), extract obtained from 2 males (2MNE) or 2 females (2FNE), or nothing (C). Different letters reflect significant Bonferroni adjusted comparisons. Shown are means (plus SEs, $N=48$)

($H=0.07$, $df=1$, 61, $P=0.79$; $H=0.42$, $df=1$, 84, $P=0.52$; Fig. 3F).

In contrast, male newts at Binghamton University were significantly attracted to females and three to four males, and significantly avoided MNE, but not FNE. As the breeding season waned, male attraction to males and females declined, and MNE and FNE avoidance concurrently increased, as predicted (Fig. 3A, B, C, D). However, only MNE and female by season portion interactions were significant (Table 2). Sample sizes for FNE and three to four males were smallest for each breeding-season portion (Fig. 3A, D), which could explain the lack of interactions. The greatest sex difference in the magnitude of MNE avoidance occurred as the non-breeding season approached (breeding season waned), with males avoiding MNE more than females (Fig. 3C, E). Finally, like the Binghamton University population, the Nuthatch Hollow population revealed significant overall male avoidance of MNE (Wald_{1, 350}=12.94, $P<0.001$) and a significant increase in avoidance of MNE as the breeding season waned, represented by a MNE by season portion interaction (Wald_{4, 350}=14.46, $P=0.006$; Fig. 4).

Fig. 3A–F Field capture rates of male and female *Notophthalmus viridescens* in the presence and absence of conspecifics and conspecific tissue extracts (MNE male newt extract, FNE female newt extract) during various portions of the breeding season (numbers before the colon are the month and after the colon are which half of the month). Male captures are only from Binghamton University’s Nature Preserve, but due to low capture rates, female captures are from both the Binghamton University and Nuthatch Hollow populations. Too few females were captured in FNE and FNE control traps to present graphically. Numbers in parentheses are sample sizes for control and treatment, respectively. Shown are means (plus or minus SEs) of trap locations that captured newts. See text for details

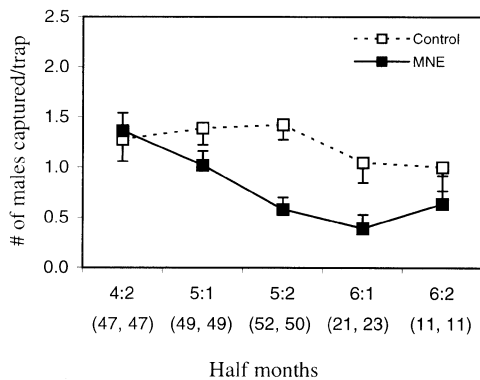
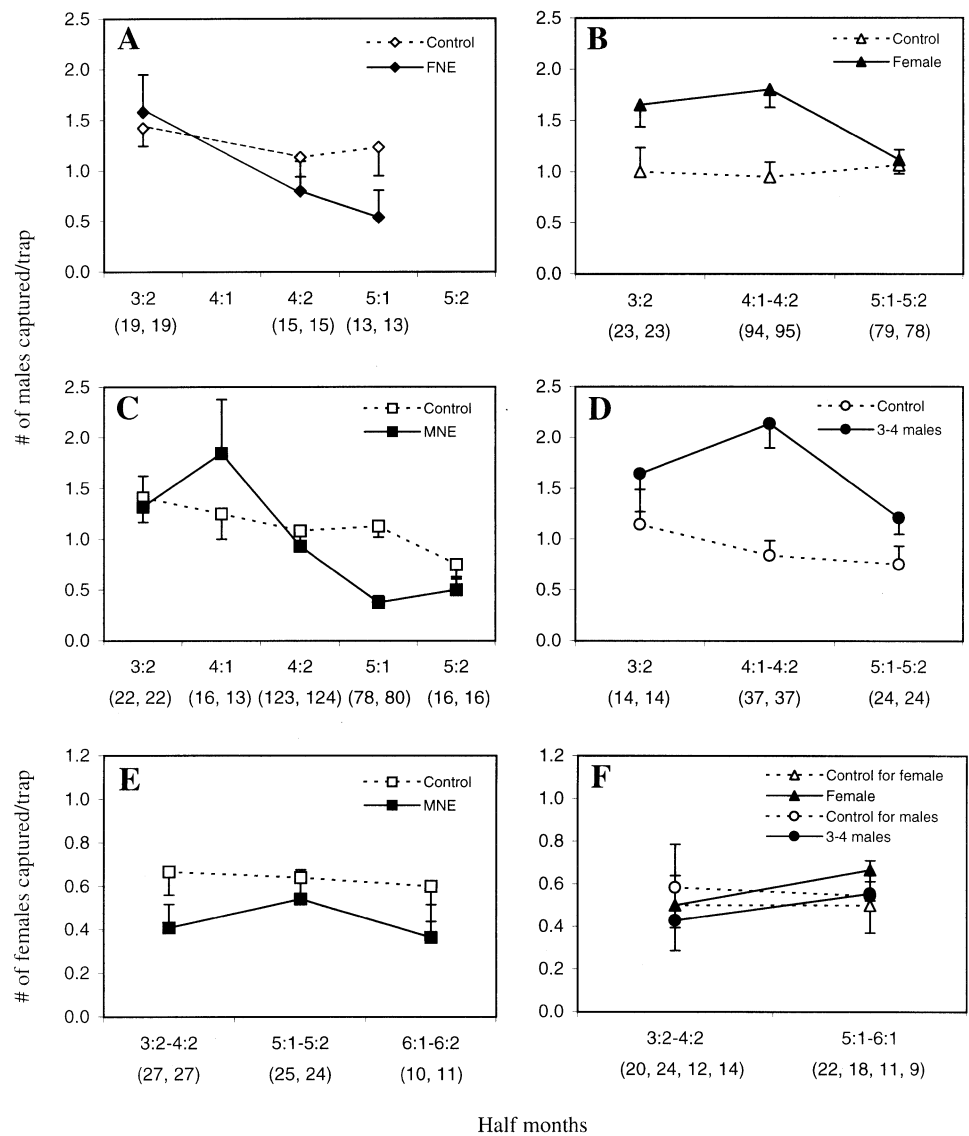


Fig. 4 Capture rates of male *Notophthalmus viridescens* at Nuthatch Hollow in the presence and absence of MNE (male newt extract) during various portions of the breeding season (numbers before the colon are the month and after the colon are which half of the month). Numbers in parentheses are sample sizes for control and MNE, respectively. Shown are means (plus or minus SEs) of trap locations that captured newts. See text for statistical analyses

Discussion

As hypothesized, male newts at Binghamton University and Nuthatch Hollow showed no avoidance of MNE early in the breeding season, but significantly increased their avoidance later in the breeding season. During the estimated peak of the breeding season (April) in both the laboratory and field, males were attracted to conspecifics and did not avoid conspecific extracts, while females were indifferent to conspecific odors and MNE. For the most part, in both the meta-analysis and Nuthatch Hollow snap-shot experiment, when males were significantly attracted to a specific sex and number of conspecifics, they did not avoid extracts from the same sex and number of conspecifics. The above results were quite robust since they were consistent between populations and years, from laboratory to field experiments, and from Nuthatch Hollow snap-shot experiment to meta-analyses.

The most surprising results of our study were that, during the breeding season, females were indifferent to males but did not avoid male extracts. Although our male treatments were obtained from at least two males, our results are consistent with those of Dawley (1984), who demonstrated that female *N. viridescens* are indifferent to the odor of a single male. However, in the newt genus *Triturus*, females are attracted to a courting male (Belvedere et al. 1988 cited in Kikuyama et al. 1999), and in the Japanese newt, *Cynops pyrrhogaster*, a female-attracting pheromone released by males has been isolated and characterized (Kikuyama et al. 1999). Source males were courting in the above studies, demonstrating female attraction to males, but were not courting in studies showing female indifference (including ours), and thus one possible explanation for these conflicting reports and female indifference to males but no avoidance of their extracts is that female-attracting substances are male-courtship (transmitted between sexual partners at short distances) rather than sex pheromones (acting at long distances; Arnold and Houck 1982). Macerated males would release both alarm substances and female attractants, which could offset one another and account for female indifference to male extracts.

In contrast to the unexpected responses of females, male responses to both females and males were generally expected and consistent with male-biased sex ratios in newts (e.g., Chadwick 1944; Hurlbert 1969; Gill 1978a, b; Verrell and Halliday 1985; estimated at 2:1 in our population), placing demands on males for intense mate search. Males were attracted to female odor only during the breeding season, which would facilitate locating mates. Contrary to the unvarying female response, male attraction to females declined through the breeding season, most likely because cumulative female insemination consistently reduced the number of receptive females and, consequently, the benefit of mate search. In *Triturus vulgaris*, the old-world relative to *N. viridescens*, females are unresponsive to males for up to 20 days after insemination (Verrell 1984), and sexual activity occurs mainly at the beginning of the breeding season (Verrell and McCabe 1988). Similar declines in activity have also been documented in other *N. viridescens* populations as the breeding season fades (Harris et al. 1988).

Male attraction to multiple male conspecifics during the peak of the breeding season may be a second, but indirect, strategy for locating females, since males often aggregate around receptive females forming "hot spots" of newt reproductive activity (personal observation). Attraction to multiple males declined as the breeding season waned, most likely because attraction to females declined, consequently making multiple males a less reliable indirect cue of a receptive female. Attraction to multiple males is consistent with other breeding-season studies on *N. viridescens* (Park and Propper 2001); however, ours is the first to confirm male attraction to multiple conspecifics in the field.

Most importantly, we believe our study has three major implications for future alarm-chemical research.

First, researchers should attempt to exclude the possibility that individuals avoid highly concentrated conspecific odors prior to drawing the conclusion that avoidance of chemicals from injured conspecifics or heterospecifics is an antipredator adaptation. It is likely that damaged tissue releases greater quantities of conspecific odors than undamaged tissue (e.g., an entire organism's supply in a well-masticated prey) and, consequently, the vicinity of an injured conspecific may be avoided because it is perceived as a highly competitive environment. Ruling out conspecific avoidance as an explanation for avoidance of damage-release cues requires demonstrating indifference or attraction to odors of dense patches of conspecifics (highly concentrated conspecific odors) rather than showing indifference or attraction to odors from a small number of conspecifics (e.g., Chivers et al. 1996, 1997; Rohr and Madison 2001a, b). This study indisputably excludes the conspecific avoidance hypothesis because, as the breeding season waned, macerated males were repulsive while increasing numbers of male newts induced greater male attraction rather than avoidance.

Future alarm-chemical research should also carefully consider the time of year prey are tested since our study supports the hypothesis that attraction to sexual pheromones within macerates counteracts alarm-cue avoidance only during the peak of the breeding season. This result is consistent with other studies that show seasonally dependent responses to alarm substances (Smith 1973; Jacobsen and Stabell 1999) and, more specifically, with the finding that attraction to gravid-female odor compromises male newt avoidance of alarm chemicals (Rohr and Madison 2001b). Interestingly, offsetting responses to sexual and alarm pheromones released from depredated newts suggest that injured or even dead conspecifics indirectly pose a greater threat to males during the breeding season than live conspecifics because depredated conspecifics may draw males to the vicinity of a foraging predator. While our data suggest that the change in response to conspecific odors induced the seasonal change in response to conspecific extracts, it is also plausible that a seasonal increase in foraging activity of a newt predator selected for newts that were sexually active before its foraging peak and thus minimized the costs of offsetting responses to sexual and alarm pheromones.

Finally, this is the first study to reveal gender-dependent responses to chemical mixtures released from damaged amphibians (see Mirza et al. 2001, for example in fish), and thus emphasizes the importance of considering gender of prey used to obtain and test alarm chemicals. Male newts avoided alarm chemicals more intensely than females during field trials at the end of the breeding season (the beginning of non-breeding season) and during laboratory trials in the non-breeding season, indicating that the gender of prey in alarm-chemical studies should be considered even during non-reproductive periods. Reliance on chemical cues to locate mates (Cogălniceanu 1994) may have predisposed male newts to greater chemical sensitivity at all times of the year, potentially accounting for the observed sex difference.

Males of many salamander species, during both the breeding and non-breeding seasons, have larger vomeronasal organs (VNO) than females (Dawley 1998); larger VNOs are hypothesized to have greater chemical sensing acuity (Hildebrand and Shepherd 1997) and, most importantly, VNOs can be used to detect chemical cues from both mates and predators (Miller and Gutzke 1999). The likely smaller VNO of females may account for the difficulty of detecting significant female responses to treatments in our study, and has potential for explaining widespread male-biased sex ratios caused by higher female mortality in red-spotted newts (Hurlbert 1969), and perhaps in other salamanders. Future studies should assess the generality of offsetting responses to sexual pheromones and alarm chemicals, whether VNO sexual dimorphisms reflect sex differences in sensitivity to multiple chemical cues and, most importantly, the consequences that both of these phenomena may have on seasonal and sex differences in predation rates.

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