ON TEMPORAL VARIATION AND CONFLICTING SELECTION PRESSURES: A TEST OF THEORY USING NEWTS

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Abstract. Most studies that examine conflicting selection pressures hold resources and risks constant, despite their ubiquitous fluctuation. Since little is known about the consequences of neglecting this variation, we examined the temporal response of male red-spotted newts, *Notophthalmus viridescens*, to conflicting female pheromones and damage-release alarm chemicals signaling predation. After a single exposure in both the laboratory and field, males were attracted to female odor and avoided conspecific alarm chemicals. Response to these combined cues depended on time after exposure, with males initially avoiding, and then being attracted to, the cue combination. This response shift was due to the resource and risk declining at different rates, and female odor accelerating male recovery from antipredator behavior. In the laboratory, males suppressed activity when exposed to alarm chemicals alone but increased their activity when female odor was added. Iterative exposures through the breeding season revealed that, as male mate search activity declined, male avoidance of alarm chemicals increased, but alarm chemical production appeared unchanged. Thawing dates differed between ponds of the same and different populations, which offset levels of mate search activity and consequently alarm chemical avoidance. As a result, simultaneous pond surveys made it appear as though there was geographic variation in reproductive and predator-avoidance behaviors. However, when thawing dates were aligned, the time courses of reproductive and predator-avoidance behaviors for the ponds coincided, demonstrating that observed site differences were predominantly due to different behavioral onsets, which would have gone overlooked had the larger temporal scale not been considered. These results indicate that temporal variation can be easily mistaken for geographic variation in behavior, increasing the potential for data interpretation errors. These studies underscore the importance of considering temporal variation when examining conflicting selection pressures.

Key words: amphibians; antipredator behavior; balancing conflicting demands; chemical cues; geographic variation; mating behavior; newts; Notophthalmus; *recovery rate.*

INTRODUCTION

Resources and predators coexist, often requiring prey to manage the conflicting pressures of resource acquisition and predator avoidance (Sih 1992, 1997). When two demands cannot be satisfied simultaneously, optimality theory predicts that evolved responses should be an optimal balance of the opposing demands (Milinski and Heller 1978, Sih 1980), requiring that the response to one demand depends on the response to the other (Horat and Semlitsch 1994). Whether prey, in general, balance resource acquisition and predator avoidance remains controversial (Horat and Semlitsch 1994). However, understanding trade-offs between conflicting selection pressures is critical because they closer simulate natural conditions where multiple cues are present, could influence population and community dynamics (Sih 1994, Lima 1998), and play a central role in the adaptationist view of trait evolution (Krebs and Davies 1991).

Despite the constant fluctuation of resources and risks in nature, the majority of studies considering prey responses to conflicting demands have used fixed resources and risks (Sih 1997, Lima and Bednekoff 1999, Sih et al. 2000). This begs the question what are the consequences of overlooking temporal variation in resources and risks when studying responses to conflicting selection pressures? This question has only recently received considerable attention. The theoretical and empirical validation of the ''risk allocation hypothesis'' (Lima and Bednekoff 1999, Sih and McCarthy 2002) now provides evidence that to fully understand behavioral responses to resources and risks, we need to know both the average intensity and temporal variation of the behavior. Below we further explore the importance of understanding temporal variation when studying conflicting selection pressures.

We came up with two theoretical, but seemingly common, scenarios where potential errors in data interpretation could arise from not considering temporal variation in resources and risks. In scenario one (Fig.

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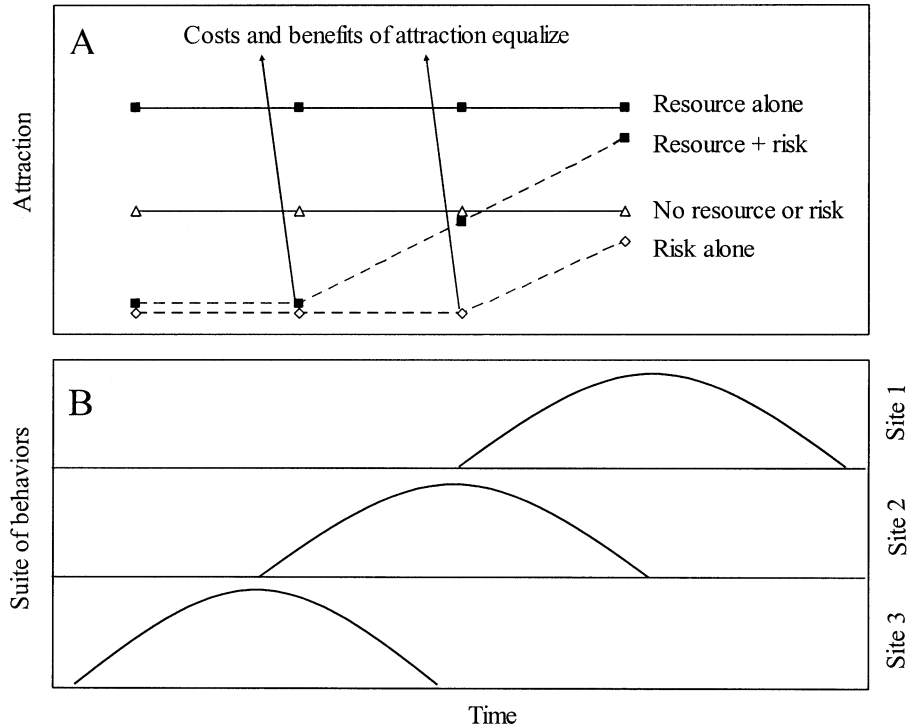
Fig. 1. Theoretical scenarios considering prey response to the presence and absence of a resource and risk (A) when the risk is initially great but declines more rapidly than the resource and (B) when this resource and risk emerge asynchronously across sites.

1A), "the recovery rate scenario," we consider the time it takes for prey exposed to a risk to return to their pre-threatened state, referred to as recovery time (Sih 1997), and do so in the presence and absence of a resource. Since it is unlikely that risks and resources coexist at the same magnitudes and decline at the same rates, the risk in this scenario is initially great (overriding the resource), but declines much more rapidly than the resource. Based on optimality theory, the costs and benefits of attraction should equalize sooner when a resource accompanies a risk than when a risk is unaccompanied by a resource. If using a "snap-shot" or "overall averaging" approach rather than considering the response through time, any response to the resource–risk combination can be obtained depending on the time of observations or the duration of the experiment. In scenario two (Fig. 1B), "the multiple site scenario," we consider the suite of responses to resources and risks on a larger spatiotemporal scale. These behaviors emerge asynchronously at these sites, making it appear as though prey respond differently at the three locations at all times, even though response types, durations (length of expression, width of curves), and magnitudes (intensity of expression, height of curves) are identical across sites.

While these potential errors seem profound, so could be the logistical costs of incorporating temporal complexity into experiments to avoid these errors. It is only through comprehensive empirical studies that we can assess whether the theoretical benefits outweigh the additional time, energy, and resources required to add this complexity. Consequently, we tested the likelihood of committing the above errors by examining mate search and predator avoidance in the red-spotted newt, *Notophthalmus viridescens*. In a static experiment, female pheromones compromised male newt avoidance of damage-release alarm chemicals signaling predation (Rohr and Madison 2001*b*), but male response to temporal variation in risk remained unstudied. Thus, to test the merits of the recovery rate scenario, we used a full factorial design to quantify laboratory and field responses of male newts to the presence and absence of female odor (the resource) and conspecific alarm chemical (the predation risk) at various times after stimulus presentation. To test the multiple site scenario, we considered male response to these same conflicting cues through the breeding season in three ponds that thaw at different times, anticipating that these thawing differences would offset breeding season emergence and responses to alarm chemicals. We expected to empirically verify both scenarios above and to demonstrate that inaccurate conclusions would have been drawn if temporal variation in behavior was not considered.

METHODS

Experiment 1: Laboratory recovery rate experiment

Experiment 1 tested the recovery rate scenario in the laboratory. Adult eastern red-spotted newts were col-

lected from a private pond in Broome County, New York, USA, in fall 1998. No more than 40 same-sex newts were placed in cattle troughs with 567 L of aged tap water and simulated vegetation. Newts were maintained at 18°C on a 14:10 light:dark photoperiod and fed a liberal diet of chopped earthworms and freeze-dried blood worms weekly.

Newts were tested using the trough and release cage design described by Rohr and Madison (2001b). Twelve test troughs (101 × 11.4 × 6.4 cm) were marked with cross lines creating four equal subdivisions, were filled with 3.5 L of aged tap water (at 20°C), contained a release cage (21 × 7.6 × 6.4 cm) at their centers, and were covered with clear plastic on both sides of the cage to isolate the system from air movements in the test room. Twelve males in breeding condition were chosen arbitrarily, and each was randomly placed in a trough that would receive a treatment that the male had not experienced previously. Newts acclimated in release cages for 75 min (suggested previously to be adequate; Rohr and Madison 2001b) before test solutions were added. Test substances included conspecific alarm chemical and female odor. Conspecific alarm substance was obtained using the methods of Woody and Mathis (1997), except that three entire males rather than only newt skin were homogenized (in dechlorinated tap water) and filtered to more closely simulate a natural predation event. We collected female pheromone by taking aliquots from a bowl that contained 600 mL of aged tap water and two captive females for 2 d (both 52 mm, snout–vent length [SVL]).

The test solution and an equal volume of aged tap water were injected simultaneously at randomly assigned, opposite ends of the trough through small holes in the plastic cover. There were four test solutions: female odor alone (F), macerated male newt extract alone (MNE), the first two treatments combined (F + MNE), and a control treatment of aged tap water (C). We prepared test solutions with either 3 mL of alarm chemical, 6 mL of female rinse water, and, if necessary, aged tap water to bring all solutions to 9 mL. Three replicates of the four treatments were run each day for 10 d, producing a sample size of 30 for each treatment. Cages were slowly lifted 5 min after solutions were injected, and then the quadrant that each newt occupied was recorded every minute for 30 min.

Experiment 2: Field recovery rate experiment

Experiment 2 tested the recovery rate scenario in the field. Male newt extract was prepared from whole males as described for Experiment 1. This extract and dechlorinated tap water were added separately in 5-mL aliquots to cellulose sponges (1 × 2.5 × 4 cm), which were frozen in Ziploc bags, according to Woody and Mathis (1997). Thirty-six minnow traps (8-mm wire mesh cylinder with funnel ends; 40 cm long and 23 cm diameter) were placed 1 m from shore and a minimum of 5 m apart at the Side Pool of Binghamton Univer-

sity's Nature Preserve (Broome County, New York, USA). We carried MNE and control water sponges on ice and 18 female newts in individual Petri dishes (containing wet paper towel) to the Side Pool where we sequentially visited and randomly placed one treatment (F, MNE, F + MNE, C) into each trap. Each female was placed into an opaque, perforated plastic container (0.47 L) tied to the center of each trap to prevent visual detection, and the same female was used at each trap location, so that differences in capture rates between F and F + MNE could not be attributed to female differences. Females were maintained as the males were in Experiment 1, except that they were held individually in plastic containers (0.47 L).

The 36 traps were divided into three arrays of 12 traps, each of which consisted of three complete sets of the four treatments, and were checked after each of three, randomly selected time intervals: 50, 100, and 150 min. As a result, each trap received each treatment checked after each time interval only once, requiring 12 d to complete the experiment. The number of male and female newts and fish caught in each trap was checked daily between 1200 and 1500 hours during mid-May 2000. Traps were left open when trials were not being conducted.

Experiment 3: Response through the breeding season at multiple sites

Experiment 3 tested the multiple site scenario in the field. The methodology of Experiment 2 was used with a few exceptions. First, the study simultaneously took place at three sites: the Main Pond and Side Pool of Binghamton University's Nature Preserve and Nuthatch Hollow pond (Broome County, New York, USA). The Main Pond and Side Pool were considered a single population because they shared a border (a beaver dam) crossed by newts (J. R. Rohr, *personal observation*). Nuthatch Hollow was a fishless pond ~1.5 km from and 150 m higher in elevation than the other two sites and therefore was considered a separate population. The Main Pond thawed first probably because it was not shaded by the mountain immediately to the south of the pond, it had minimal tree cover, and it had a large shallow littoral zone. The Side Pool consistently thawed 2–3 wk later than the Main Pond (J. R. Rohr, four years of *personal observation*), because of mountain shading, greater tree cover, and a deeper littoral zone. Nuthatch Hollow consistently thawed a few weeks after the Side Pool (J. R. Rohr, four years of *personal observation*), probably because of its higher elevation.

We removed F and F + MNE treatments and used 24 rather than 36 traps at each site, due to the proposed complexity of Experiment 3 and because we knew that male attraction to females and mate search activity peaked and then declined during the breeding season (Rohr et al. 2002). Despite removing the resource from this experiment, we submit that there still are conflict-

ing demands because the substantial activity required for males to search for females remaining in the ponds would increase encounter rates with, and conspicuousness to, predators (Skelly 1994). Once traps had received each treatment checked after each time interval, we repeated the 6-d trapping process 3 wk after the starting date of the previous trial. There were four of these 6-d trials, referred to as ''rounds.'' Newt activity remains low from the end of the breeding season until the start of the next breeding season (Harris et al. 1988). Thus, to minimize the number of newts sacrificed, we stopped trapping at sites once the mean number of newts captured per trap dropped below 0.25. As a result, we initially prepared four batches of MNE, each created from three adult male newts in breeding condition (mass of newts per batch: 1 = 10.65 g, 2 = 9.96 g, 3 = 8.87 g, 4 = 8.64 g each in 533 mL of dechlorinated water). Each site received equal quantities of all four batches, and prior to each round, batches were randomly assigned to traps. Batches 1–4 were only assigned to rounds 1 and 2, and two additional batches were required (5 = 9.35 g, 6 = 8.93 g) for rounds 3 and 4. Finally, we applied firm pressure to the abdomen of captured females to assess whether eggs were exuded from their cloacas. This method was used successfully by Verrell and Halliday (1985) and Verrell and McCabe (1988) to evaluate timing of ovulation in smooth newts, *Triturus vulgaris*.

Statistical analyses and hypotheses

All statistical analyses were conducted using Statistica software (Statsoft 1998), and alpha values were not adjusted for the number of multiple comparisons because the intent of these studies was to assess types of data interpretation errors that could have been made had temporal variation not been considered. For Experiment 1, we used a repeated-measures multivariate analysis of variance (MANOVA) to evaluate the effects of independent factors, female odor and MNE (presence and absence) in the first and last 15 min of trials, on the dependent variables, location (quadrant location relative to test solution) and activity (number of lines crossed; Johnson and Wichern 1998). After MANOVA, we used univariate analysis of variance (ANOVA) on each response variable to interpret patterns uncovered by the MANOVA. Based on the results of a Box-Cox test (Neter et al. 1996), our activity data were inverse and arcsine transformed to meet the assumptions of parametric analyses. No transformations were necessary for the location data. We expected males to avoid MNE alone and be attracted to female odor alone for the entire experiment. However, we expected males to initially avoid F + MNE but, as the concentration of MNE and therefore risk decreased (due to diffusion), males were expected to move up the female odor gradient in the second 15 min. MNE was expected to suppress activity to decrease encounters with predators,

and female treatment was expected to increase activity to increase encounters with potential mates.

For Experiment 2, we compared the number of newts captured using Poisson regression (PR) in the Generalized Linear Model (GLZM; McCullagh and Nelder 1989) with the identity canonical link and main effects: female (present or absent), MNE (present or absent), and time (50, 100, 150 min). Hypotheses analogous to those used in the laboratory experiment were applied to the field experiment.

Experiment 3 had missing cells because we stopped trapping at some sites to minimize the number of newts sacrificed. Since there are no nonparametric procedures to accurately deal with missing cell designs, and since our data did not meet parametric assumptions, we used a combination of parametric and nonparametric statistics. We used the 2/3 power transformation suggested by McCullagh and Nelder (1989) to approximate symmetry from a Poisson distribution and then used ANOVA with Type VI sums of squares (recommended for missing cells designs; Hocking [1996]) to parametrically analyze the largest factorial model possible. We excluded round 4 from parametric analyses because this model unambiguously provided the most probability values without further sacrificing model size. For nonparametric tests, we used Poisson regression in GLZM. We hypothesized that thawing differences would result in the asynchronous onset of mate search activity at the sites, that mate search activity and alarm chemical avoidance would be correlated inversely as described previously (Rohr et al. 2002), and due to this correlation, thawing differences would also indirectly produce asynchronous emergence of alarm chemical avoidance.

For Experiments 2 and 3, the data provided a good fit to the chosen GLZ model (deviance scores between 0.8 and 1.5; McCullagh and Nelder 1989), and for all analyses using GLZM, we chose to test for significance of effects using the Wald statistic (analogous to a least squares estimate), rather than likelihood ratios, due to its ease and efficiency of computation (Dobson 1990). We also used independent samples chi-square tests to assess whether there were treatment differences in number of traps containing newts rather than in number of newts per trap. This procedure controlled for conspecific attraction, which could inflate a small effect size and therefore account for treatment differences. Fish captures were not expected to influence newt captures because the dominant fish at these sites, bluegill sunfish, *Lepomis macrochirus*, are not reported as predators of adult newts (Hurlbert 1970, Kesler and Munns 1991). However, as a precautionary measure, we compared fish captures between treatments using a Friedman ANOVA and tested for a correlation between fish and newt captures using a Spearman rank correlation test (Siegel and Castellan 1988).

TABLE 1. Results of MANOVA and ANOVAs from Experiment 1 and Poisson regression from Experiment 2.

	Experiment 1									Experiment 2		
	MANOVA			ANOVAs						Poisson regression		
				Location			Activity					
Effects	df	F	P	df	F	P	df	F	P	df	Wald	P
Female	2	5.526	0.005	1	10.916	0.001	1	0.156	0.693	1	25.352	<0.001
MNE	2	7.134	0.001	1	5.059	0.026	1	8.972	0.003	1	20.167	<0.001
Time	2	17.793	<0.001	1	3.407	0.067	1	31.905	<0.001	2	31.719	<0.001
Female × MNE	2	3.225	0.043	1	0.163	0.688	1	6.286	0.014	1	0.667	0.414
Female × time	2	0.581	0.561	1	1.054	0.307	1	0.138	0.711	2	5.768	0.056
MNE × time	2	2.916	0.058	1	5.837	0.017	1	0.076	0.783	2	0.582	0.747
Female × MNE × time	2	1.338	0.266	1	2.624	0.108	1	0.054	0.817	2	0.431	0.806
Error	115			116			116					

Notes: MANOVA tested for effects of female odor (presence or absence) and male newt extract (MNE; presence and absence) on response variables, male location (location) and activity (the number of lines crossed), in the first and second 15 min (time) of 30-min laboratory trials, and ANOVAs were conducted for each response variable. Poisson regression tested for effects of female odor (presence or absence) and MNE (presence and absence) on the number of newts captured per trap after three time intervals (50, 100, or 150 min). Experiments were conducted in natural ponds in Broome County, New York, USA.

RESULTS

For Experiment 1, MANOVA revealed strong main effects of female, MNE, and time on male response and a significant interaction between female and MNE (Table 1). ANOVA revealed that males significantly avoided MNE, were significantly attracted to female odor, and as hypothesized, initially avoided F + MNE followed by attraction (Table 1, Fig. 2A). Male newts significantly increased their activity throughout trials in response to all treatments, but unlike newt location, change in activity over time did not differ between treatments (Fig. 2B, Table 1). Males were significantly less active when exposed to treatments with, than without, MNE. Activity in the presence of female odor did not significantly differ from activity in treatments with-

out female odor, but female odor did significantly increase male activity in the presence of MNE.

In field experiments, female capture rates were low (0.042 and 0.035 females/trap for Experiments 2 and 3, respectively) and did not differ between treatments for Experiment 2 (C = 4, F = 5, MNE = 3, F + MNE = 6, N = 108) or 3 (Main Pond, C = 2, MNE = 2, N = 144; Side Pool, C = 10, MNE = 7, N = 216; Nuthatch, C = 16, MNE = 9, N = 288; total: $\chi^2_2 = 2.17$, $P = 0.14$), so we examined total newts captured for simplicity. Preliminary analyses revealed significant main effects of treatment regardless of whether we controlled for conspecific attraction to captured newts, and there was no correlation between fish and newt captures and no significant difference in the number of fish captured between treatments.
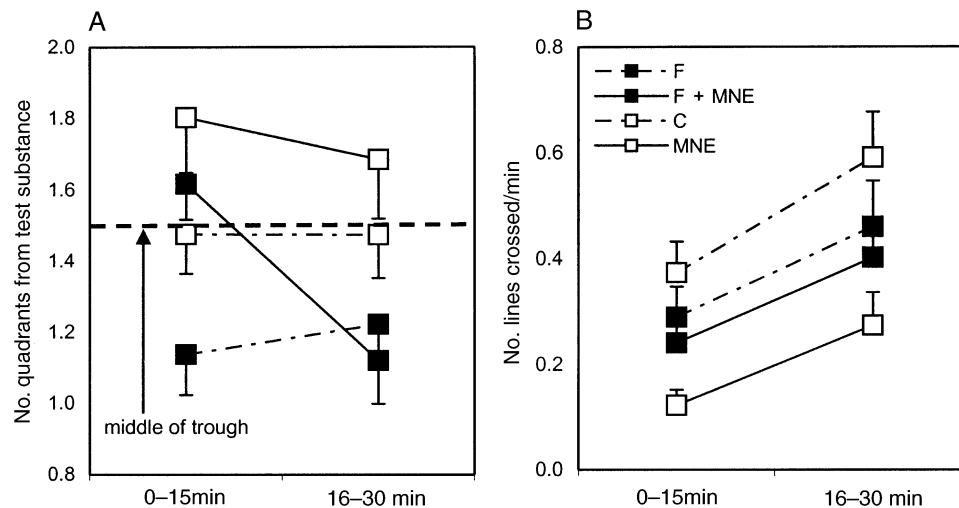

FIG. 2. (A) Avoidance and (B) activity of male newts responding to female odor (F), male newt extract (MNE), their combination (F + MNE), and control water (C) during the first and second 15 min of laboratory trials. Symbols in (A) reflect the distance from the quadrant in which the test solution was initially injected (means − 1 SE), and symbols in (B) reflect the associated number of lines crossed during trials (means + 1 SE).
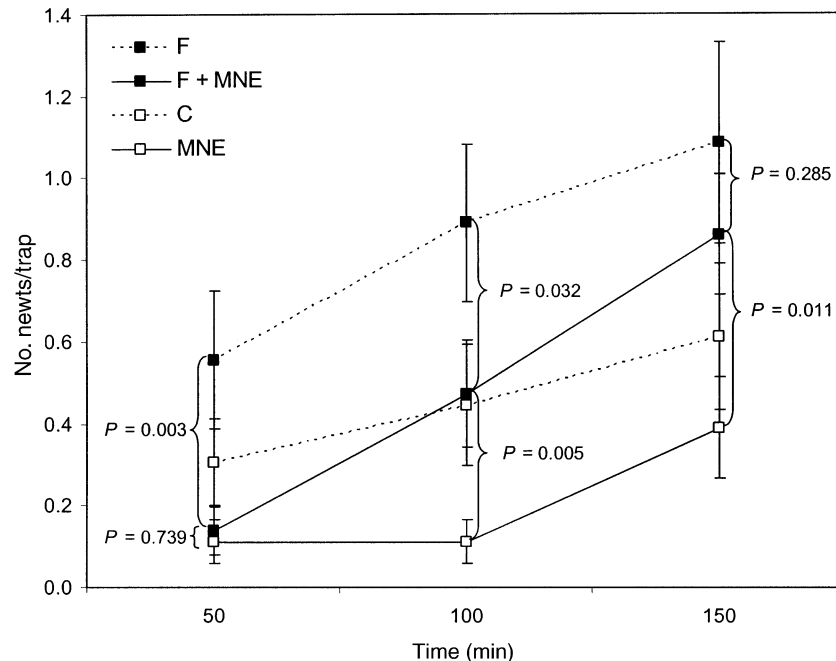
FIG. 3. Number of newts captured per trap containing a female (F), male newt extract (MNE), their combination (F + MNE), or control water (C), after 50, 100, and 150 min (means ± 1 SE). Probability values are provided for bracketed comparisons and were calculated using Poisson regression. Experiments were conducted in natural ponds in Broome County, New York, USA.

For Experiment 2, newts avoided MNE treatments, were attracted to female treatments, and capture rates increased over time (Fig. 3). There were no significant full model interactions (Table 1), but there was a significant partial model interaction. Traps containing F + MNE had a significantly greater increase in capture rates from 50 to 100 min than traps containing MNE alone (PR, Wald$_{1,140}$ = 4.80, $P <$ 0.05). Thus, like the laboratory experiment, newts recovered more rapidly from antipredator behavior induced by MNE when a female was present. As a result, if the three time intervals were examined independently we would have received three different results. At 50 min, F + MNE traps caught significantly fewer newts than F traps, but did not differ from MNE traps; at 100 min, capture rates in F + MNE traps were statistically intermediate to those in F and MNE traps; and at 150 min, F + MNE traps caught significantly more newts than MNE traps, but did not differ from F traps.

For Experiment 3, all males and females captured were in breeding condition, but females did not exude eggs when squeezed. We did not set Main Pond traps for rounds 3 or 4 or Side Pool traps for round 4 because mean number of newts captured per trap dropped below 0.25 the previous round (Fig. 4). There was no significant difference in number of newts captured among batches 1–4 (PR: Wald$_{3,860}$ = 5.03, P = 0.170) or between batches 5 and 6 (PR: Wald$_{1,430}$ = 0.49, P = 0.484), and same batches elicited avoidance in some rounds but not others (Fig. 4).

Parametric and nonparametric analyses produced similar results. For both analyses, main effects were significant, indicating that different numbers of newts were captured between sites, rounds, treatments, and times, and significant interactions were dominated by the site factor, suggesting that different behaviors were expressed at different rounds, times, and treatments at at least two of the three sites (Table 2, Fig. 4). Notably, mean capture rates per trap declined steadily throughout rounds, consistent with decreasing mate search activity. In addition, every site had a round by time interaction, as seen by the decreasing slope of treatment lines from one round to the next (Table 2, Fig. 2), consistent with decreasing attraction to conspecifics caught in traps. The one exception to these trends was at Nuthatch Hollow from round 1 to 2, which was probably due to trapping at this site prior to newts reaching peak mate search activity. As predicted, MNE avoidance increased as mate search activity (capture rates) declined, and there was only significant MNE avoidance when capture rates were relatively low. MNE avoidance was probably not detected when capture rates reached their lowest points due to minimal statistical power.

As in Experiment 2, had time intervals, in this case rounds, been examined independently we would have received different results. If the experiment was only conducted during round 1, only newts at the Main Pond would have exhibited significant MNE avoidance, and newts at the Side Pool would have had the greatest
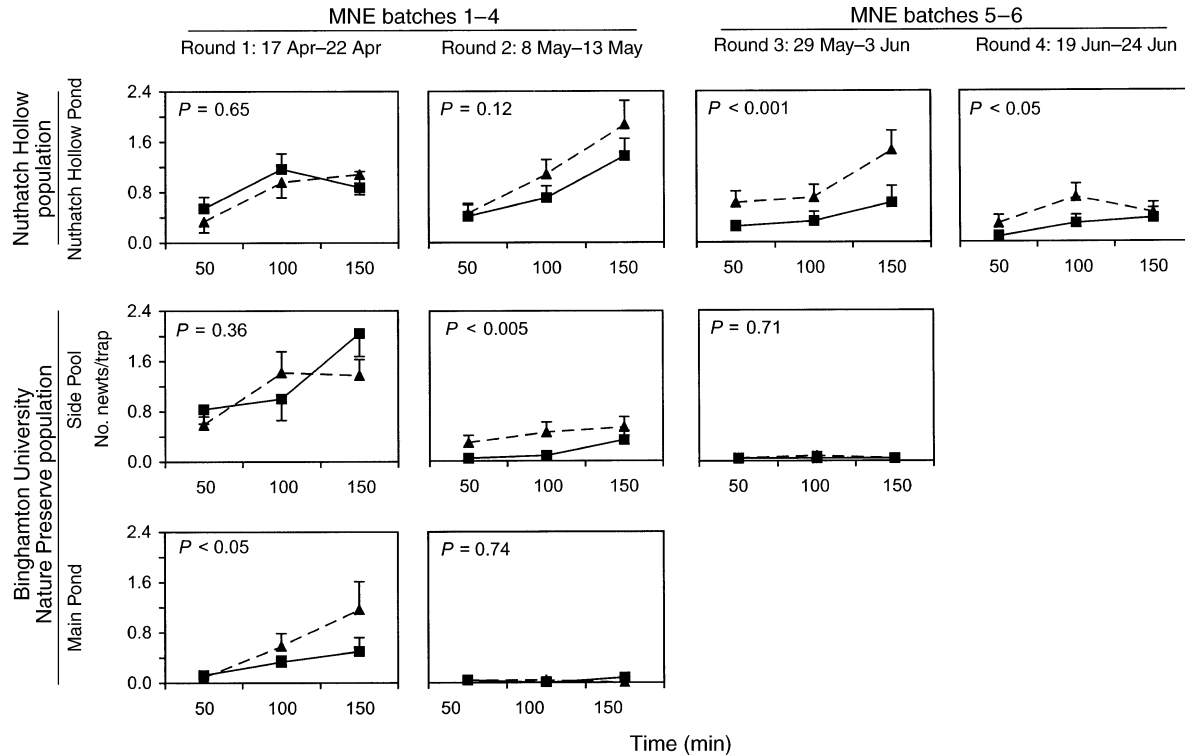
FIG. 4. Number of newts captured per trap containing either a control sponge (triangles and dashed lines) or male newt extract sponge (MNE; squares and solid lines) after 50, 100, and 150 min during four different times of the newt breeding season (rounds 1–4; means ± 1 SE). Traps were set at three sites (Nuthatch Hollow, Side Pool, Main Pond) within two populations (Nuthatch Hollow and Binghamton University's Nature Preserve), and six different batches of MNE were used in the experiment. Probability values for the main effect of MNE are provided for each site within each round and were calculated using Poisson regression. Trials were not conducted at sites once the mean number of newts captured per trap dropped below 0.25 to minimize the number of newts sacrificed to obtain MNE.

capture rates/activity. Had traps only been checked during round 2, only newts at the Side Pool would have shown significant MNE avoidance, and newts at Nuthatch Hollow would have had the greatest activity. The same logic can be applied to subsequent rounds.

Although the statistics for each individual round indicate site disparities, the data along the diagonals of Fig. 4 appear similar, suggesting that different behavioral onsets at these three sites could explain interactions containing the site factor. We tested this a priori prediction by rerunning the analyses with the diagonals of Fig. 4 aligned, approximately equivalent to aligning site thawing dates. All interactions containing the site factor became nonsignificant (last column of Table 2), indicating that there were no significant site differences in newt behaviors after aligning thawing dates.

Since the data support newt behaviors being offset temporally, we vertically aligned the diagonals of Fig. 4 to look for differences in female capture rates and sex ratios of captured newts. We assumed that round 4 of Nuthatch Hollow, 3 of the Side Pool, and 2 of the Main Pond were "late" in the breeding season and labeled backward in time from this round (Table 3). Females did not significantly avoid MNE when com-

bining all rounds (C = 28, MNE = 18; N = 648, χ^2 = 3.400, P = 0.065). The greatest number of males and females captured per trap were synchronized during the "early-middle" portion (E-M) of the breeding season (Table 3), and consequently this period is considered the "peak" of reproductive activity. The sex ratio of captured newts was significantly less male biased "late" (L) in the breeding season than all other times (E, χ^2 = 9.27, P = 0.002; E–M, χ^2 = 10.62, P = 0.001; L-M, χ^2 = 8.95, P = 0.003), which were not significantly different from one another (df = 2, χ^2 = 0.351, P = 0.839; Table 3). This abrupt change in sex ratio was due to a decrease in male capture rates (df = 3, χ^2 = 100.18, $P < 0.001$; Table 3), while female capture rates remained constant (L–M vs. L: the only portions of the breeding season tested at all sites, χ^2 = 0.010, P = 0.920).

DISCUSSION

Recovery from antipredator behavior within the breeding season

Male newts were attracted to females, and they avoided (and decreased their activity in response to)

TABLE 2. Results of parametric and nonparametric ANOVAs for the effects of site (Nuthatch, Side Pool, Main Pond), round (four different times of the breeding season), time (50, 100, 150 min), and male newt extract (MNE; present or absent) on newt captures in traps.

	Parametric analyses			Nonparametric analyses								
	Rounds 1–3			Rounds 1–2			Rounds 2–3			Rounds 1–2 MP, 2–3 SP, and 3–4 N		
	N, SP, MP			N, SP, MP			N, SP			N, SP, MP		
Factor	df†	F	P	df	Wald	P	df	Wald	P	df	Wald	P
Site	1	29.777	<0.001	2	137.879	<0.001	1	123.630	<0.001	2	14.782	0.001
Round	1	42.334	<0.001	1	49.054	<0.001	1	21.918	<0.001	1	21.310	<0.001
Time	...	...	...	2	101.030	<0.001	2	52.293	<0.001	2	11.497	0.003
MNE	...	...	...	1	3.332	0.068	1	23.027	<0.001	1	8.900	0.003
Site × round	3	23.273	<0.001	2	48.899	<0.001	1	0.123	0.725	2	1.024	0.599
Site × time	2	3.102	0.045	4	14.076	0.007	2	29.301	<0.001	4	2.235	0.693
Round × time	2	2.166	0.115	2	8.423	0.015	2	7.215	0.027	2	8.038	0.018
Site × MNE	1	1.671	0.196	2	0.348	0.840	1	5.479	0.019	2	2.426	0.297
Round × MNE	1	2.597	0.107	1	2.741	0.098	1	0.014	0.907	1	3.670	0.055
Time × MNE	...	...	...	2	4.120	0.127	2	1.996	0.369	2	0.594	0.743
Site × round × time	6	4.124	<0.001	4	20.529	<0.001	2	1.556	0.459	4	2.788	0.594
Site × round × MNE	3	3.666	0.012	2	12.746	0.002	1	4.438	0.035	2	0.013	0.994
Site × time × MNE	2	3.427	0.033	4	7.798	0.099	2	3.415	0.181	4	0.873	0.928
Round × time × MNE	2	0.299	0.741	2	0.590	0.744	2	1.451	0.484	2	1.531	0.465
Site × round × time × MNE	6	0.703	0.647	4	6.200	0.185	2	0.753	0.686	4	1.839	0.765

Notes: Parametric analyses were conducted using type VI sums of squares on only rounds 1–3 because too many effects could not be unambiguously estimated (signified by ellipses) using the full factorial model. Nonparametric analyses were conducted using Poisson regression. The column examining different rounds at each site is our best estimate of aligning similar times of the breeding season at each site. MP, SP, and N refer to the Main Pond, Side Pool, and Nuthatch sites, respectively.

† Error df = 1104.

conspecific tissue extracts, consistent with previous results (Dawley 1984, Ducey and Dulkiewicz 1994, Marvin and Hutchinson 1995, Woody and Mathis 1997, Rohr and Madison 2001a, b). The increasing activity over time in response to all laboratory treatments may have been due to lifting the release cage; Cochran and Redmer (1992) demonstrated that terrestrial newts were initially motionless when their refuge rocks were lifted, followed by a gradual resumption of activity. Surprisingly, newt location but not activity showed a risk by time interaction indicating that types of simultaneously occurring antipredator behaviors induced by the same stimulus can change differently over time, previously referred to as dissociated responses (Madison et al. 1999).

As with the findings of Rohr and Madison (2001b), male behavior was consistent with an underlying trade-off between mate search and predator avoidance. An intermediate response to female plus MNE relative to the two odors alone indicates that predation risk compromised mate search, and that male newts took greater risk in the presence of a female, potentially increasing their susceptibility to predation. This intermediate response did not occur early or late in experiments, suggesting that males responded to changing concentrations of alarm chemical. Magnitude of antipredator responses is often correlated positively with concentration of chemical cues signaling predation (e.g., Petranka 1989, Horat and Semlitsch 1994, Loose and Dawidowicz 1994, McKelvey and Forward 1995, Van

TABLE 3. Female-to-male (F/M) ratios of newts captured in traps and the number of females captured per trap (F/trap) at each site (Nuthatch Hollow, Side Pool, Main Pond) at four different times of the breeding season (early, early-middle, late-middle, late).

Site	Early	Early-middle	Late-middle	Late	Total
F/M ratio					
Nuthatch	5/114 (0.044)	7/135 (0.052)	4/95 (0.042)	9/41 (0.220)	25/385 (0.065)
Side Pool	...	11/163 (0.067)	6/38 (0.158)	0/7 (0.000)	17/208 (0.082)
Main Pond	...	...	2/65 (0.031)	2/3 (0.667)	4/68 (0.059)
All sites	5/114 (0.044)	18/298 (0.060)	12/198 (0.061)	11/51 (0.216)	46/661 (0.070)
F/trap					
All sites	5/144 (0.035)	18/288 (0.063)	12/432 (0.028)	11/432 (0.025)	46/1296 (0.035)

Notes: Rounds have been realigned to estimate similar times in the breeding season. Ellipses represent trials that were not conducted for ethical reasons. See *Results* for details.

Buskirk and Arioli 2002). Therefore, avoidance early in trials may have been due to highly concentrated alarm chemical reflecting imminent threat. As the alarm chemical became diluted, the perceived predator threat probably decreased, which could release newts to resume mate search.

By quantifying duration of newt responses to conspecific alarm chemical, we were able to indirectly estimate its persistence in different field environments and consequently add to the dearth of information about chemical cue persistence in nature (Dodson et al. 1994, Chivers and Smith 1998). Since capture rates in F + MNE traps were not statistically different from C traps after 100 min, we estimate that, at this site, the biologically relevant persistence of this alarm substance in the presence of a female was slightly greater than 100 min. In the absence of a female, alarm chemical avoidance ceased at ~150 min, suggesting that persistence of MNE alone was somewhat greater than 150 min. In general, this persistence was relatively moderate in length, considering that voles, *Clethrionomys glareolus*, avoided predator odors for several days (Jedrzejiewski and Jedrzejiewska 1990), while tadpoles, *Bufo americanus*, only avoided conspecific alarm cues combined with food for 5–8 min (Petranka 1989). Clearly, our estimates are not highly precise, and more accurate persistence measurements are needed.

The more transient persistence of alarm chemical combined with female odor relative to alarm chemical alone is congruous with optimal foraging experiments. In the studies of Petranka (1989) and Sih (1992), recovery from antipredator behavior was faster in the presence of food. Only one study other than our own examined recovery in the presence of a potential mate. Kålås et al. (1995) demonstrated on both theoretical and empirical grounds that following a threatening stimulus, male Great Snipes, *Gallinago media*, returned to leks containing females sooner than to leks without females. We, like the authors of these studies, propose that prey species balanced the conflicting demands of resource acquisition and predator avoidance and that recovery was more rapid in the presence of a resource because the costs and benefits of avoidance equalized sooner. The growing number of studies verifying increased recovery from antipredator behaviors in the presence of a resource suggests that this may be a general prey response.

Antipredator behavior through the breeding season

Our data also support males balancing predator avoidance and reproductive potential on the large temporal scale of the breeding season. Male newts did not avoid conspecific tissue extracts at Nuthatch Hollow or the Side Pool at what we regard as the prime of the breeding season, but significantly increased their avoidance as the breeding season waned. Various factors could not account for this temporal change in response to MNE. Captured newts and fish did not sig-

nificantly influence treatment differences, and females showed no significant avoidance of (or temporal change in response to) MNE, making it unlikely that any of these explanations alone could have produced the change in male response. While many ostariophysan fish produce less alarm chemical in breeding condition (Smith 1992), a seasonal change in production could not explain our results because within each site the same batches of MNE produced avoidance in some rounds but not others. Therefore, the temporal change must be due to behavioral plasticity in male response to alarm chemicals.

This behavioral change was likely induced by a decrease in female availability later in the breeding season, reducing the benefit for males to disregard MNE. Male mate search activity, attraction to conspecifics (Rohr et al. 2002), and female sexual receptivity (Massey 1988) decline later in the breeding season, consistent with the proposed reduction in mating probability and the results of this study. Female sexual receptivity decreases sharply at the onset of oviposition for the smooth newt, *T. vulgaris* (Verrell and McCabe 1988), and a similar phenomenon may account for the observed abrupt decline in male activity in the "late" portion of the breeding season. Unfortunately, we were unable to squeeze eggs from captured females to assess timing of ovulation. We were also unable to forcefully exude eggs from females ovipositing in the laboratory, suggesting that this methodology to assess oviposition is not effective for *N. viridescens*.

Consequences of the failure to consider temporal variation in resources and risks

Both scenarios presented in the introduction were verified empirically, demonstrating the imperative of considering resource and risk variation across temporal scales. In Experiments 1 and 2, any conclusions from responses recorded at any one time, rather than through time, could have propagated misconceptions about newt behavior, since they may have suggested a more fixed rather than time- or concentration-dependent response. In Experiment 3, newts within each round behaved differently at each site, but the only means of discovering that responses were actually similar across sites was to examine the larger temporal scale, which exposed behavior offset by the approximate thawing differences.

The upshot of this finding from Experiment 3 is that temporal variation in behavior can be easily mistaken for spatial variation, increasing the likelihood of Type I and II errors. A Type I error would have occurred had we claimed geographic variation in type, magnitude, or duration of behavior, since the only significant source of variation was in emergence. A Type II error may have occurred had we not analyzed the bordering subpopulations separately. This would have increased the variation in response to alarm chemicals making it more difficult to demonstrate that the population, as a

whole, avoided injured conspecifics. Although ecotypic variation in behavior across large spatial scales is common (Beattie 1985, Foster and Endler 1999), variation in behavior of prey between local subpopulations is rarely considered. In fact, asynchronous breeding season emergence likely explains previously reported discrepancies in MNE avoidance at the Main Pond and Side Pool (Rohr and Madison 2001*a*, *b*). We found no mention of the specific errors above in Foster and Endler's (1999) recently published text on evolutionary mechanisms of geographic variation in behavior (but see Carroll and Corneli [1999] for general discussion of errors). Neither could we find any studies in which temporal variation was ruled out experimentally as an explanation for geographic variation, with the exception of studies that demonstrated that genetic differences were responsible for the observed behavioral differences. Consequently, past studies should be reexamined in light of these potential errors.

One specific example in which the consideration of temporal variation can offer insight is the controversy over the balancing hypothesis. Horat and Semlitsch (1994) argue that balancing conflicting demands requires that both demands be integrated into a behavioral decision represented by a statistical interaction between the demands and that many studies have not demonstrated a balancing of demands because prey responses to the demands were additive rather than multiplicative. However, as seen in Experiments 1 and 2, the response to resource–risk combinations can be identical first to the risk-alone response and then grade into the resource-alone response, which can produce opposing resource–risk interactions early and late in experiments. If behavior is averaged over time, as often is the case, these opposing interactions can counteract, making it erroneously appear that independent mechanisms were at work and the demands were not balanced. Therefore, in cases where temporal response changes were not quantified, prey may be balancing demands when an interaction between the demands is absent. Consequently, temporal response changes should be considered more often, and the requirements for balancing conflicting demands should be expanded to include resource- or risk-by-time interactions.

CONCLUSIONS

We have demonstrated that behaviors within newt mating and predator avoidance systems are correlated and that there are considerable costs to neglecting temporal variation for these systems. Furthermore, we contend that this phenomenon is not unique to newts since temporal variation and behavioral correlations are common (see Lima and Dill 1990). Therefore, to fully understand the entirety of a response, it may be necessary to quantify its intensity, emergence, and duration at various spatiotemporal scales, in addition to quantifying other types of simultaneously occurring responses that may be potential covariates. Although managing this complexity may not always be practical, behavioral ecologists should make an effort to incorporate this complexity into their experiments when possible or at least acknowledge the risks of not doing so.

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