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Dryness increases predation risk in efts: support for an amphibian decline hypothesis

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Abstract One hypothesis for amphibian declines is that increased dryness attributed to global climate change exposes amphibians to greater biotic threat and, consequently, greater mortality. But, little is known about behavioral responses of terrestrial amphibians to dry conditions alone or in combination with biotic threats. We used field observations and laboratory experiments to test the response of efts (terrestrial juveniles) of the eastern red-spotted newt, *Notophthalmus viridescens*, to separate and combined desiccation and predation risks. When only at risk of desiccation, efts moved into shade, traveled down slope, decreased activity, and adopted water-conserving postures. Efts also significantly reduced the rate of water loss by huddling and were attracted to chemical cues from conspecific efts but not from conspecific adults. Thus, efts have a variety of behaviors that reduce the risk of dehydration associated with climate change. When faced only with a predation risk, represented by adult and eft newt tissue extracts (alarm chemicals), efts reduced their activity and avoided alarm cues from both sources. When exposed to combined desiccation and predation risks, efts were less active than when exposed to either risk separately and avoided adult tissue extracts, but not eft extracts. These results suggest that under dry conditions, conspecific tissue extracts contain both attractive (huddling) and repulsive (predator-related) chemical components that induce offsetting behavioral responses. This is the first study to demonstrate moisture-dependent responses to conspecific rinses and alarm substances, underscoring the importance of considering environmental moisture and animal hydration in studies examining responses to conspecific odors and/or alarm chemicals. These results support the hypothesis that elevated dehydration risk may compromise anti-predator behavior and exacerbate amphibian population declines.

Keywords Chemical cues · Huddling · Conflicting demands · Individual variation and consistency · Salamander

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Introduction

Various hypotheses have been proposed for recent widespread amphibian declines and extinctions (Blaustein and Kiesecker 2002). An underlying cause for many of these declines is thought to be drier conditions associated with global climate change (Pounds et al. 1999; Kiesecker et al. 2001). Climate models incorporating global warming trends predict drier and warmer summer weather, increased and prolonged hydrologic variability in mid latitudes of the United States, and intensified El Niño/Southern Oscillations (Loaiciga et al. 1996; Wetherald and Manabe 1999). Drier weather is a particular threat to amphibians because their integument is highly permeable, providing little resistance to evaporative water loss, even when in contact with moist substrates and at high relative humidity (Spotila 1972).

While decreased moisture alone could induce amphibian declines, evidence suggests that this variable may work in concert with others to produce more precipitous declines (Pounds and Crump 1994; Kiesecker et al. 2001). For instance, warming trends accelerate drying in shallow lakes and ponds, increasing the risk of egg/larval dehydration and parasitism, and this combined threat seems to explain amphibian declines in the United States Pacific Northwest better than either threat alone (Kiesecker et al. 2001). A synergism between desiccation and biotic risk has also been hypothesized to cause higher mortality in the more terrestrial life-history stages of juvenile and adult amphibians (Pounds and Crump 1994; Pounds et al.

1999). For example, Pounds and Crump (1987) found that changes in habitat use in response to unusually dry weather in Costa Rica increased risk of attack by parasitoid flies.

Even though effective and plastic behavioral responses to changes in moisture should mitigate the adverse effects of dryness, little is known about behavioral responses to dehydration in terrestrial amphibians or about the ecological consequences of their moisture limitations (Putnam and Hillman 1977; Feder 1983). Even less well understood are synergisms between environmental change and biotic interactions. In fact, Blaustein and Kiesecker (2002) recently implored for investigations to improve our understanding of context-dependent interactions between environmental change at local, regional, and global scales and modifications of biotic interactions because they argue that this complexity is one of the only general explanations for amphibian declines. In response to this entreaty, we examined the behavioral response of recently metamorphosed efts (terrestrial juveniles) of the red-spotted newt, *Notophthalmus viridescens*, to desiccation and predation risks, considered both separately and combined. Although red-spotted newts are not known to be in decline, their response to combined desiccation and biotic threats could provide insight into declines of other amphibian species.

Efts are excellent subjects for these studies because their high surface-to-volume ratio [~2–4 cm snout-vent length (SVL), ~0.5–2 g] likely makes them susceptible to dehydration, they remain in the surface humus and leaf litter during dry weather, they are active during the warmest times of the year (late spring, summer, and early fall), they travel substantial distances above ground during formal migrations and inter-habitat wandering, and they restrict their activity to periods of high moisture (Hurlbert 1969, 1970; Healy 1975). They also are common in mid latitudes of the United States (Petranka 1998), which are predicted to have more frequent dry periods (Loaiciga et al. 1996; Wetherald and Manabe 1999). Despite this susceptibility to dehydration, little is known about eft behavior under xeric conditions.

Field evidence also suggests that adults and efts face considerable predation (Shure et al. 1989; Rohr and Madison 2002), despite possessing a potent neurotoxin (tetrodotoxin; Brodie 1968). The predators on newts in nature have not been identified, but the type of field predation and predation under laboratory settings suggest that skunks, raccoons, bullfrogs, and turtles are their putative predators in the northeastern United States (Hurlbert 1970; Shure et al. 1989; Rohr and Madison 2002). Furthermore, the eft life-history stage may be at high risk of predation because of inexperience with terrestrial predators during emigration and because they are probably unfamiliar with the location of terrestrial refugia during emigration and inter-habitat wandering.

We used field observations and laboratory experiments to determine how efts respond to shade, slope, conspecifics, and predation cues in wet and dry conditions. We predicted that efts exposed to desiccation risk would seek shade, huddle with conspecifics, decrease activity, adopt water-conserving postures, and travel down slope because these responses should reduce evaporative water loss or increase the likelihood of moving toward moist habitats. We predicted that efts exposed to predation-related chemicals released from injured conspecifics would reduce activity and exhibit avoidance because these are typical responses to damage-release alarm chemicals that reduce encounters with predators responsible for injury (Chivers and Smith 1998). Furthermore, heterospecific damage-release chemicals are not avoided by adult newts (Marvin and Hutchison 1995), but conspecific alarm chemicals reduce adult activity (Rohr and Madison 2001) and induce avoidance by adults and efts (Rohr et al. 2002a, 2002b), but adults do not avoid

When exposed to simultaneous predation and desiccation risks, we hypothesized a tradeoff between avoidance of conspecific alarm chemicals and attraction to conspecifics. Dryness often induces conspecific attraction in amphibians, facilitating huddling to reduce evaporative water loss (e.g., Gehlbach et al. 1969; Heinen 1993). During the breeding season, conspecific tissue extracts of adult newts contain both attractive sex pheromones and repulsive predation-related alarm chemicals that have offsetting effects (Rohr et al. 2002a). Thus, analogous to adult newt extract during the breeding season, eft tissue extract during dry spells should contain chemical cues eliciting both conspecific attraction (huddling) and avoidance responses (anti-predator response), resulting in "indifference" to conspecific tissue extracts under dry conditions. This experimental outcome would provide evidence that increased dryness exposes amphibians to enhanced biotic threats, which in turn could stimulate amphibian declines.

Materials and methods

Field observations

After an evening of rain in August, efts were observed at sunrise (wet and cool) and again at 1100 hours (drier and warmer) as they attempted to cross a closed road while emigrating from the Binghamton University Nature Preserve pond, Broome County, N.Y. We recorded the direction of eft movement (uphill or downhill), whether they were at the curb closest to or farthest from the pond, and whether they were huddled with conspecifics, defined as physical contact with another eft. We expected efts to be non-huddled, facing uphill, and at the curb farthest from the pond during early morning and huddled, facing downhill, and at the curb closest to the pond during late morning, because these changes in behavior should facilitate reducing surface area, moving toward water, and returning to the pond as conditions become drier and warmer through the morning.

General methods for experiments 1–4

Approximately 100 efts were collected along the road mentioned above following field observations. Efts were kept refrigerated at 10 °C immediately after capture and between experiments. Two weeks before their use in each experiment, efts were placed at 20 °C with a natural photoperiod (window light) in 15-cm plastic petri dishes lined with moistened filter paper. During this period, efts were fed flightless *Drosophila* sp. *ad libitum*, and their filter paper was changed every 3 days. Irregularities in sample size among experiments were due to occasional eft escape or death during these 2 weeks.

Efts for each experiment were randomly chosen from a pool of approximately 100 efts and then placed in the center of petri dishes containing filter paper. Experiments 1–3 had both wet and dry petri dishes. The wet dishes were thoroughly misted with dechlorinated tap water before eft distribution. All experiments occurred between 1300 and 1500 hours and were conducted under natural light, except for experiment 1. After an experiment, efts were returned to the refrigerator and kept moist for a week before the next pre-experimental period. We are unaware of any non-damaging method to sex immature newts, and consequently the sex of efts in all experiments was unknown.

Experiment 1

To determine whether efts exhibit negative phototaxis under dry conditions, 22 efts were placed singly in the center of 11 wet and 11 dry petri dishes. All dishes had a double layer of black construction paper placed over half the top and were visually isolated with construction paper strips placed around each dish. The orientation (right and left) of the shaded side was randomly stratified among the 22 dishes. The location of efts in dishes was recorded after 1 h. Overhead fluorescent lighting rather than natural light was used because it was more uniformly distributed.

Experiment 2

To test for eft huddling in dry conditions, 69 efts were distributed among 12 wet and 11 dry petri dishes until each dish had three efts. After 1 h, we recorded whether each group of three efts (triads) had individuals positioned as singlets (S, no efts touching one another), a doublet (D, two efts touching one another), or a triplet (T, three efts touching one another).

Experiment 3

To determine whether efts exhibit negative geotaxis and huddling in response to dry conditions, 13 wet and 13 dry petri dishes were placed on a 13° incline, and triads were placed in each dish. Wet and dry dishes were positioned randomly on the table, as was the direction of the inclination for each dish. Huddling (T, D, S) and position (upper/lower half) were recorded after 1 h.

Experiment 4

To determine whether huddling reduces eft water (mass) loss during dry periods, 20 dry petri dishes lined with filter paper were weighed; half then received triads and half received single efts, and the dishes containing the efts were then weighed (±0.001 g) every 30 min for 150 min.

Experiment 5

To assess eft response to concurrent desiccation and predation risks, we collected approximately 100 larval newts from a private pond in Chenango County, N.Y., during August 2000 and 2001. Larvae were placed in a 64-l aquarium containing dechlorinated tap water and simulated vegetation, and the aquarium was inside a 567-l cattle trough filled with 2 cm of dechlorinated tap water. The larvae were maintained in a controlled environment chamber at 20 °C on a 14:10 h light:dark cycle and fed freeze-dried brine shrimp and blood worms ad libitum. Each morning we gathered metamorphosed larvae that crawled out of the aquarium and fell into the cattle trough, and we randomly assigned these efts to one of two 32-l aquaria (in the same chamber) containing paper towels for cover. Efts were fed flightless *Drosophila* sp. ad libitum and their tanks were thoroughly misted twice daily.

To obtain rinse from efts, 10 efts (mean SVL mm ± SE, 2000: 19.8±0.64; 2001: 19.3±0.63) with gill remnants were placed in 200 ml of dechlorinated tap water for 48 h. Efts with fully absorbed gills were not chosen because they frequently climbed out of the water. This water was then filtered through glass wool, and 10-ml aliquots were immediately frozen. To acquire alarm chemicals from efts, the same efts used for the rinse were decapitated, macerated in 200 ml of dechlorinated tap water, and filtered through glass wool and 10-ml aliquots were immediately frozen. Freezing does not appear to influence the usefulness of conspecific alarm signals for the red-spotted newt (Marvin and Hutchison 1995). Rinse and extracts from an aquatic adult male newt (SVL 48 mm, 2000; 49 mm, 2001) were obtained in the same manner, using the same volumes of water. Chemicals from adults were obtained to determine whether eft responses were specific to their own life-history stage. Control water was handled, divided, and frozen as in all other treatments, and treatments were thawed just before trials. These procedures were used for trials conducted in 2000 and 2001.

Tests of preferences were again conducted in petri dishes. Two filter-paper semi-circles (11 cm diameter) were placed in each of 30 petri dishes (10 cm diameter × 1.5 cm height) and separated by a 3-mm gap that minimized treatments from mixing between the halves. One semi-circle in each dish received 1.0 ml of control water, and the other semi-circle received 1.0 ml of control water or 1.0 ml of rinse or extract from eft or adult newts. The moistened filter paper was pressed along the sides and bottom of each petri dish because efts can climb the sides of dishes. Since all test dishes received 2 ml of fluid, we could not have dry and wet dishes as in the previously described experiments. Instead, we stopped misting one holding tank 48 h before the start of trials, which should be well within natural climatic fluctuations in moisture. During this time, multiple efts were frequently found intertwined in the “dry,” but not in the “wet,” holding tank. Five hours before trials, 15 efts from each tank were transferred to individual, labeled, 10-cm petri dishes containing a piece of paper towel, and we misted only petri dishes containing efts from the tank that we continued to mist. Efts exposed to dry and wet conditions 48 h before the start of the experiment will be referred to as “dry” or “wet” efts, respectively.

Petri dishes were randomly positioned and oriented (right or left) on a grid placed on the floor of a test room. Efts were removed from their home dish and placed in the center of their randomly assigned test dish, and their movements were recorded for 100 min under complete darkness using an infrared video camera. Two trials were conducted per day. Trials took place between 1400 and 1800 hours in September of 2000 and 2001. Individuals were exposed to each treatment once in random order. All efts were returned to their home dishes after testing, and the same testing procedures were repeated the following day. This procedure produced a sample size of 15 for each treatment each year.

We recorded the side occupied by each eft every 2 min for the last 80 min of each recording period, which provided an initial 20-min habituation period. If efts straddled the gap between semi-circles, the location of their snout was used as the criterion of choice (Marvin and Hutchison 1995). We also recorded the number of times efts (excluding their tails) crossed the 3-mm gap of their petri dish in the first 80 min, providing an estimate of activity.

We argue that the most adaptive response of efts under dry conditions should be attraction to non-injured conspecifics to facilitate huddling with another moist object, but avoidance of injured conspecifics because there should be alternative objects away from predation risk that could be used to reduce surface area. Exposing individuals to all treatments in random order allowed us to test for correlations in individual activity and location responses across treatments. These correlations provide an estimate of the abundance of individuals that respond “most adaptively” to predation risk under dry conditions and the degree of variability in response upon which selection can act. Both are important for determining the likelihood that the population can evolve to minimize the risk of predation in impending drier environments.

Statistical analyses

Chi-square goodness-of-fit tests (chi-square GOF) were used for field observations to test for eft directional preference, and for laboratory experiments 1–3 to test for position in shade or light, on the upper or lower half of inclined dishes, and to test whether huddling occurred (T + D vs S) with conspecifics. Chi-square contingency tables (chi-square 2×2) were used for field observations to compare eft behavior between the two observation periods and for laboratory experiments 1–3 (Yates corrected) to compare eft response to wet and dry conditions (Siegel and Castellan 1988). For experiment 4, we used repeated measures ANOVA to assess the effect of huddling on eft mass over time. The location data in experiment 5 were bimodally distributed, and, consequently, the data were dichotomized into efts that avoided or were attracted to each treatment. Rinse or extract "avoidance" was scored if $\geq$21 of the 41 observations were on the control side. We then used binomial regression with a log-log canonical link in Statistica's (Statsoft, Inc., 1998 version 5.5A) Generalized Linear Model to test for effects of treatment (rinse or extract), source of treatment (eft or adult), and moisture condition (dry or wet). The log-likelihood ratio chi-square was used to determine significance of effects (McCullagh and Nelder 1989). Binomial regression with year as a blocking variable revealed no significant main effect of, or interaction containing, year. To preserve degrees of freedom, we ran all subsequent tests excluding year as a factor. To analyze activity data from experiment 5, we used a Mann-Whitney *U* test to compare activity between dry and wet efts and a Kruskal-Wallis test to compare activity between adult and eft rinses and extracts (Siegel and Castellan 1988). Poisson regression could not be used because the number of times efts switched sides of the dish did not fit a Poisson distribution. Finally, we used Kendall coefficient of concordance (Kcc) to test for inter-individual variation and intra-individual consistency in activity and location responses (Siegel and Castellan 1988).

Results

Field observations

At sunrise, 148 efts were observed moving along the curbs of Binghamton University's Nature Preserve road. Of these, 129 were along the curb farthest from the pond, 19 were along the curb closest to the pond (chi-square GOF, $\chi^2_1=81.76$, $P<0.001$), 105 were moving uphill, 28 were moving downhill (chi-square GOF, $\chi^2_1=45.58$, $P<0.001$), and 15 were not heading in either direction. At 1100 hours the same day, 64 efts were observed; 56 were along the curb farthest from the pond, 8 were along the curb closest to the pond (chi-square GOF, $\chi^2_1=36.00$, $P<0.001$), 24 were facing uphill, 33 were facing downhill (chi-square GOF, $\chi^2_1=2.03$, $P=0.15$), and 7 were not facing in either direction. There was no significant difference in the percentage of efts along the two curbs between the two observation periods (chi-square 2×2, $\chi^2_1=0.00$, $P=0.95$), but significantly more efts were facing uphill along the road at sunrise than during the warmer 1100 hour observation period (chi-square 2×2, $\chi^2_1=24.85$, $P<0.001$). Most efts observed at sunrise were actively crawling, while most at 1100 hours were immobile and wedged along the shaded and moistened crack at the base of the curb. No efts were clumped at sunrise, but three pairs were clumped at 1100 hours.

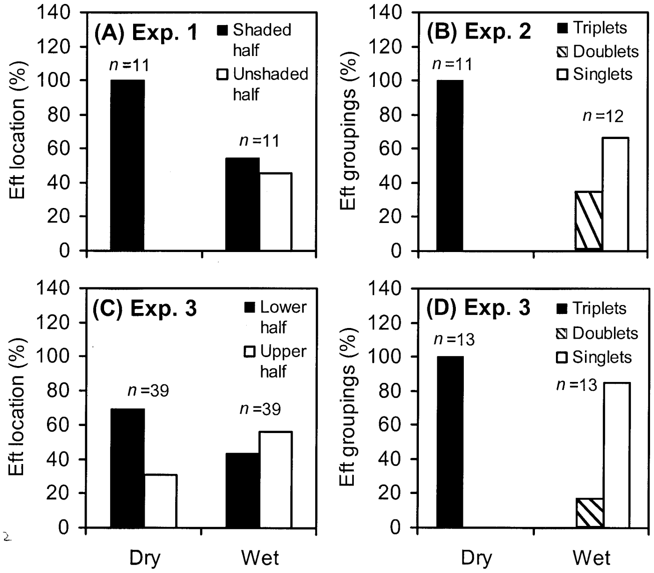

Fig. 1 Eft preference in wet and dry conditions for **A** shade, **B** huddling with conspecifics (triplets, doublets, or singlets), **C** concurrent slope direction (upper or lower half of inclined dish), and **D** huddling in the same experiment

Experiment 1

Efts in dry dishes were found under the shaded portion significantly more often than under the non-shaded portion (chi-square GOF, $\chi^2_1=11.00$, $P<0.001$), but efts in wet dishes were not (chi-square GOF, $\chi^2_1=0.09$, $P=0.76$), resulting in a significant difference in shade preference between efts in wet and dry conditions (chi-square 2×2, $\chi^2_1=4.14$, $P<0.05$; Fig. 1A).

Experiment 2

Eft triads exposed to dry conditions were found clumped (triplets and doublets) more often than as singlets (chi-square GOF, $\chi^2_1=11.00$, $P<0.001$), but triads exposed to wet conditions showed no preference for being clumped (chi-square GOF, $\chi^2_1=1.33$, $P=0.24$), resulting in a significant difference in clumping between efts in wet and dry dishes (chi-square 2×2, $\chi^2_1=8.50$, $P<0.005$; Fig. 1B). All clumping occurred against the sides of the petri dishes.

Experiment 3

The 39 efts (13 triads) subjected to an incline under dry conditions significantly preferred the lower half of the dish over the upper half (chi-square GOF, $\chi^2_1=5.78$, $P<0.001$; Fig. 1C) and to be clumped rather than in singlets (chi-square GOF, $\chi^2_1=13.00$, $P<0.001$; Fig. 1D). The 39 efts exposed to wet conditions showed no preference for the upper or lower half of the dish (chi-square GOF, $\chi^2_1=0.64$, $P=0.42$; Fig. 1C) and significantly

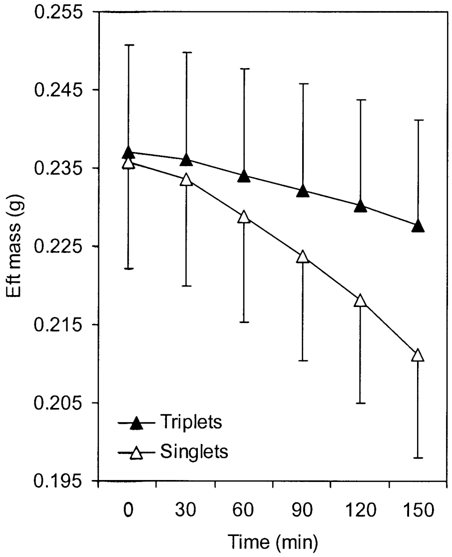

Fig. 2 Mean mass (±SE) through time for three huddled efts and a single eft (n_1=n_2=10) exposed to dry conditions

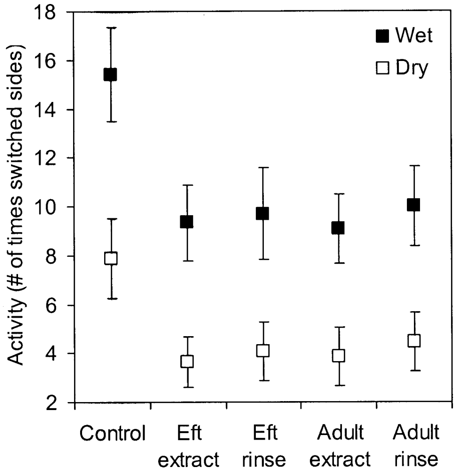

Fig. 3 Mean activity (±SE) of efts (number of times they switched sides) in test dishes with both halves containing control water or one half containing control water and the other half containing eft or adult extracts or rinses. Efts were exposed to wet or dry conditions before trials

Table 1 Repeated-measures ANOVA testing effects of eft grouping (singlets, triplets) and time on eft mass loss

Factor	*df*	*F*	*P*
Grouping	1, 18	0.180	0.677
Time	5, 90	577.008	<0.001
Grouping × Time	5, 90	115.722	<0.001

preferred to be distributed as singlets rather than clumped (chi-square GOF, χ^2_1=6.23, P<0.02; Fig. 1D). There was a significant difference in incline location between efts in wet and dry conditions (chi-square 2×2, χ^2_1=5.21, P<0.03).

Experiment 4

Under dehydrating conditions, all triads were clumped in triplets throughout the 150-min observation period, presumably resulting in the significantly lower rate of mass loss for triads compared to solitary efts (Fig. 2, Table 1). After 150 min, efts huddled in triplets lost only 4.05% (±0.23 SE) of their original mass compared to 10.64% (±0.79 SE) for the single efts.

Experiment 5

There was a significant main effect of treatment (control water, and eft and adult rinses and extracts) on eft activity (Kruskal Wallis, $H_{4,300}$=12.93, P=0.012; Fig. 3). Five treatment comparisons were made to determine where treatment differences occurred, reducing our alpha to 0.01 (Bonferroni adjustment). Efts in control water dishes were significantly more active than efts in dishes containing eft extract (Kruskal Wallis, $H_{1,120}$=7.66, P=0.006), eft rinse (Kruskal Wallis, $H_{1,120}$=8.44, P=0.004), and adult extract (Kruskal Wallis, $H_{1,120}$=8.92, P=0.003) and were nearly more active than efts in dishes containing adult rinse (Kruskal Wallis, $H_{1,120}$=6.06, P=0.014). There was no significant difference between eft activity in dishes containing eft or adult rinses or extracts (Kruskal Wallis, $H_{3,240}$=0.31, P=0.958).

Eft activity was also significantly affected by moisture. Dry efts were significantly less active than wet efts (Fig. 3; Mann-Whitney U, U=6617, N_1=N_2=150, P<0.001) and showed less variation in activity. Most dry efts also seemed to adopt classic water-conserving postures. Their limbs were tucked against their body, and their ventral surface was pressed against the substrate. Most importantly, efts exhibited a greater reduction in activity when exposed to combined desiccation and predation risks than when exposed to either risk separately (Fig. 3).

Location data revealed that extracts induced greater avoidance than rinses, that adult treatments induced greater avoidance than eft treatments, and that wet efts avoided treatments more than dry efts (Fig. 4, Table 2). When exposed to adult treatments, both wet and dry efts were indifferent to rinse and significantly avoided extract. When exposed to eft treatments, wet efts were indifferent to rinse and significantly avoided extract, while dry efts were significantly attracted to rinse and did not avoid extract (Fig. 4).

Tests for individual variation and consistency revealed that there were significant differences in activity among individuals and that individual activity was positively correlated (and thus consistent) across treatments under both wet (Kcc, W=0.568, χ^2=82.38, P<0.001) and dry (Kcc, W=0.372, χ^2=53.91, P=0.003) conditions relative to other individuals tested. In contrast to the activity data, the bimodality and bounded nature (had both upper and

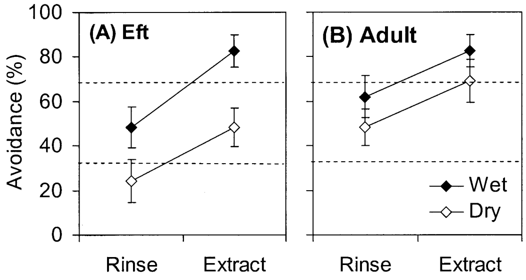

Fig. 4 Mean percent (±SE) of efts, previously exposed to dry or wet conditions, that avoided rinses and extracts obtained from (**A**) efts and (**B**) adult newts (n=30). Probability thresholds for chi-square goodness-of-fit tests are represented by the *dotted lines*. Results outside of this range demonstrate significance at P<0.05

Table 2 Results of binomial regression for effect of treatment (rinse, extract), treatment source (eft, adult), and moisture condition (dry, wet) on eft treatment avoidance

Factor	*df*	Log-likelihood ratio X^2	*P*
Treatment	1	15.083	<0.001
Treatment source	1	5.116	0.024
Moisture	1	12.017	<0.001
Treatment × Source	1	0.703	0.402
Treatment × Moisture	1	0.074	0.786
Source × Moisture	1	2.059	0.151
Treatment × Source × Moisture	1	0.014	0.907

lower limits) of the location data produced numerous tied ranks (59% and 56% of ranks for wet and dry, respectively), which greatly compromised our ability to detect correlations in location among treatments for dry (Kcc, W=0.295, χ^2=34.27, P=0.230) or wet (Kcc, W=0.237, χ^2=27.50, P=0.545) conditions.

Discussion

Response to a desiccation risk alone

In the laboratory and field, eft responses to a desiccation risk seemed appropriate and were consistent with previous amphibian studies. Dehydration occurs more rapidly in warmer temperatures (Spotila 1972), so moving under shade, where ambient temperatures are often lower, should reduce evaporative water loss. In the laboratory, efts preferred shade in dry conditions, and in the field, efts moved to the shaded crack between the road and curb as temperatures rose (late morning). Similar to efts, *Bufo marinus* and *B. valliceps* selected lower ambient temperatures in dry conditions, which significantly reduced water loss (Malvin and Wood 1991; Williams and Wygoda 1993).

Traveling down slope should bring efts to moister habitats. Once again, laboratory and field behaviors were consistent, with efts moving down slope in dry petri dishes and shifting from moving up slope in the field at sunrise (moist conditions) to facing down slope during late morning (drying conditions). We found only one anecdotal report of amphibians moving down slope in dry conditions (Keen 1984).

In contrast to downhill movement, huddling with conspecifics has been frequently reported for anurans (Heinen 1993) and caudates (Wells and Wells 1976), and in all cases aggregations were observed only in dry weather (mid- to late summer). Huddling has been reported for newly metamorphosed *Bufo americanus* (Heinen 1993) and *B. boreas* (Arnold and Wassersug 1978); subadult *Ambystoma macrodactylum*, *A. tigrinum*, and *A. maculatum*; and subadult and adult *Plethodon cinereus* and *P. glutinosus* (Wells and Wells 1976 and references therein), but our study is the first to show huddling in salamandrids. Reduced water loss of huddled efts is consistent with the findings of Gehlbach et al. (1969) and Heinen (1993), who showed that huddling reduced water loss in *A. tigrinum* and *B. americanus*, respectively. Collectively, these results suggest that conspecific attraction and huddling may be a general amphibian strategy to avoid desiccation.

Eft odor alone was sufficient to attract efts in dry conditions, which may facilitate locating huddling partners by identifying refuges containing efts. However, the finding that dehydrated efts were more attracted to chemicals from efts than from adult newts was unexpected. It is more likely that efts would be near each other than near adults because adults are only occasionally found on land (Hurlbert 1969); therefore, eft odor may be a more reliable cue for potential huddling associations.

While it is possible that conspecific attraction and huddling is anti-predator in function (Arnold and Wassersug 1978), our data support only the anti-dehydration hypothesis. If conspecific attraction decreases predation risk under moist conditions, efts should have been attracted to conspecific odors within the tissue extracts. Under dry conditions, efts should have been attracted to conspecific odor because it would decrease the risk of both desiccation and predation. Efts expressed neither of these responses.

Reduction of eft activity in response to a dehydration threat is consistent with our field observations, with greater activity of efts on forest floors after rains (Healy 1975), and with the decreased activity of other amphibians exposed to temporarily xeric conditions (e.g., Keen 1984). Decreased activity was probably a voluntary rather than involuntary behavioral change because (1) reduced capacity to move by aerobic or anaerobic means could not explain activity reductions of efts dehydrated to 80% of their standard mass (Stefanski et al. 1989); (2) no dry efts appeared to reach their critical activity point, where they lose their righting response (Gillis and Breuer 1984); and (3) many dry efts moved throughout the 80-min observation period. Because salamanders rehydrate by making body contact with moist surfaces, the reduction in eft activity would allow efts to lay prostrate, increasing body contact with moist substrates and decreasing exposed surface area (Putnam and Hillman 1977). Thus, the benefits of decreased activity are probably to assist in rehydration and prevent more extreme dehydration.

Response to a predation risk alone

Predators frequently injure prey, and thus eft avoidance of injured conspecifics may be an indirect and adaptive mechanism to avoid the vicinity of a foraging predator. Reduced activity in response to extracts corroborates the findings of Rohr and Madison (2001) and may decrease encounter rates with predators. Predators that consumed newts in the laboratory often ate both adults and efts (Hurlbert 1970). Thus, when adult newts move to terrestrial habitats during the late summer (Hurlbert 1969), efts and adults likely coexist and share predators, potentially providing a benefit for efts to respond to both injured eft and adult newts (Rohr et al. 2002b).

Response to concurrent desiccation and predation risks

The greater reduction in activity of efts when exposed to a combined desiccation and predation risk, than when exposed to either risk separately, suggests that the two risks induce non-conflicting responses that are additive at these risk levels. However, the likely detrimental upshot of a greater reduction in activity with concurrent risks is a concomitant greater reduction in foraging and growth rates. Decreased activity induced by drying conditions or predator cues has been implicated in reduced foraging success of plethodontid salamanders (e.g., Feder and Londos 1984; Stefanski et al. 1989; Maerz et al. 2001) and leptodactylid frogs (Pough et al. 1983), and these indirect, non-lethal effects may be more damaging to prey populations than mortality directly attributed to these factors (Lima 1998). For example, it is quite possible that reduced foraging could reduce immunocompetence and increase susceptibility to pathogens, which are thought to be an important contributing factor to amphibian declines (Dasak et al. 1999).

In contrast to activity, eft directional response to desiccation risk and conspecific extracts was conflicting, supporting the independence or “dissociation” of salamander activity and directional response (Madison et al. 1999; Rohr et al. 2002b). As hypothesized, efts were attracted to conspecific odors under dry conditions and avoided conspecific extracts under wet conditions, but were indifferent to eft alarm chemicals when at risk of desiccation. Because both these attractive and repulsive pheromones should be components of whole-body newt macerate, indifference to alarm chemicals in dry dishes suggests that attraction to eft odors (to facilitate huddling) compromised avoidance of alarm chemicals signaling predation.

It is likely that concurrent predation and desiccation risks occur frequently for terrestrial amphibians, but our study is the first to examine them simultaneously and to demonstrate a moisture-dependent tradeoff between conspecific odors and damage-release cues. These findings underscore the importance of considering the moisture of test environments and test animals when examining amphibian responses to conspecific odors and alarm chemicals. Although we did not quantify eft desiccation or predation rates, on average, mitigating actions were not taken for either threat when combined, suggesting greater desiccation and predation risk, which supports the amphibian decline hypothesis that increased dryness attributed to global climate change exposes amphibians to enhanced biotic threats. Abolished avoidance of alarm chemicals during dry spells is likely common for amphibians, since many species huddle in dry conditions (Wells and Wells 1976), use chemical cues to locate conspecifics (e.g., Rohr et al. 2002a, 2002b), and respond to damage-release alarm chemicals (Chivers and Smith 1998).

Individual variation in activity and directional responses

Conservation biologists are often interested in whether populations are capable of adapting to environmental change, but few quantify individual variation in behaviors that mitigate risk, despite this being the raw material on which selection acts (Caro 1998). By repeat testing of efts, we demonstrated that individual activity was correlated positively across treatments, and, because a reduction in activity should reduce the risk of both predation and desiccation, we have also demonstrated that the raw material exists for eft adaptation to gradual changes in these risks (assuming that activity is heritable).

Consistent individual variation in eft directional response to chemicals from non-injured and injured conspecifics unfortunately remains equivocal because of the nature of the data collected. However, further investigations are warranted, because if there is a positive correlation between attraction to conspecific rinses and extracts (perhaps due to pleiotropy), then, despite consistent individual variation, the presumed most adaptive mean location response under dry conditions—attraction to non-injured conspecifics but avoidance of injured conspecifics—may not be reachable because this response would require a substantial portion of individuals exhibiting a negative rather than a positive correlation. Consequently, efts may not have the phenotypic plasticity to respond most adaptively to predation risk in impending drier environments. This example illustrates that in order for conservation biologists to better predict the ability of populations to adapt to environmental change, we need more studies that determine whether consistent individual variation in response to risk exists, whether the trait is heritable, and whether the type of variation present would allow for the most adaptive peak to be reached.

Conclusions

Our results indicate that efts have a variety of behavioral responses to combat predicted increased summer dryness associated with climate change and seem to respond adaptively to chemicals representing predation risk in moist habitats. However, attraction to pheromones to facilitate huddling under dry conditions appears to offset the avoidance of predation-related conspecific alarm chemicals, resulting in ostensibly maladaptive responses to concurrent predation and desiccation risks. This suggests that increased dryness attributed to global climate change could expose amphibians to enhanced predation risk, which in turn could stimulate amphibian declines. Furthermore, efts, and perhaps other amphibians, may lack the variation to adapt to significant predation in impending drier environments. Clearly, the implications of simultaneous predation and desiccation risks for terrestrial amphibian growth and survival deserve further study.

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