

Correlated trait response: comparing amphibian defense strategies across a stress gradient

T.S. Garcia, D.J. Paoletti, and A.R. Blaustein

Abstract: Animals inhabiting complex environments often contend with multiple stressors that can select for conflicting responses. Individuals can mediate these conflicts by utilizing correlated responses across multiple traits. In aquatic habitats, larval amphibians often face conflicting, simultaneous pressures, such as ultraviolet-B (UV-B) radiation and predators. UV-B radiation and predation risk influence behavior and body color in many amphibian species, altering activity rates, refuge use, and coloration. When both UV-B and predators are present, individuals can avoid conflicts by coupling behavior with body color to form a correlated response. UV-B exposure rates vary along an elevation gradient, thus trait combinations may also vary. We quantified changes in activity rates and body color in two anuran species, the red-legged frog (*Rana aurora* Baird and Girard, 1852) (low elevations) and the cascades frog (*Rana cascadae* Slater, 1939) (high elevations), during exposure to predator chemical cues (rough-skinned newt, *Taricha granulosa* (Skilton, 1849)) and UV-B radiation. *Rana aurora* decreased activity in response to UV-B and became more cryptic over time, while *R. cascadae* coupled decreased activity rates in response to predators with dark body coloration to screen out UV-B. Both species responded with a correlated trait response, yet employed opposite strategies. This observed species difference may be reflective of differences in stress across habitats and availability of alternative defenses.

Résumé : Les animaux qui vivent dans des milieux complexes doivent souvent faire face à de multiples sources de stress qui peuvent mener à la sélection de réactions incompatibles. Les individus peuvent réconcilier ces conflits en utilisant des réactions corrélées parmi leurs multiples traits. Dans les habitats aquatiques, les larves d'amphibiens subissent souvent des pressions simultanées qui sont en conflit, telles que la radiation ultraviolette B (UV-B) et les prédateurs. La radiation UV-B et la prédation influencent toutes deux le comportement et la coloration du corps chez plusieurs espèces d'amphibiens, entraînant une modification des taux d'activité, de l'utilisation des refuges et de la coloration. Lorsqu'il y a à la fois de l'UV-B et des prédateurs, les individus peuvent éviter le conflit en associant le comportement et la coloration corporelle pour produire une réaction corrélée. Comme les taux d'exposition à l'UV-B changent le long d'un gradient altitudinal, la combinaison de traits peut aussi varier. Nous avons mesuré les changements de taux d'activité et de coloration corporelle chez deux espèces d'anoures, la grenouille à pattes rouges (*Rana aurora* Baird et Girard, 1852) (basses altitudes) et la grenouille des Cascades (*Rana cascadae* Slater 1939) (hautes altitudes), lors d'expositions à des signaux chimiques de prédateurs (le triton rugueux, *Taricha granulosa* (Skilton, 1849)) et à la radiation UV-B. *Rana aurora* ont réduit leur activité en réaction à l'UV-B et sont devenues plus cryptiques avec le temps, alors que *R. cascadae* ont associé leur taux réduit d'activité en réaction aux prédateurs à une coloration plus foncée pour filtrer l'UV-B. Les deux espèces réagissent par une réponse corrélée de traits, tout en utilisant des stratégies opposées. Cette différence spécifique que nous observons peut refléter des différences de stress entre les habitats et la disponibilité de plusieurs moyens de défense de rechange.

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Introduction

In nature, animals are exposed to a suite of environmental factors simultaneously. Individuals can attempt to mediate potential conflicts in optimal response by utilizing correlated responses from multiple traits. These paired responses may result in the combination of behavioral and physiological traits. For example, in aquatic habitats, larval amphibians face conflicting selection pressures from concurrent environ-

mental stressors. Two of these stressors, sunlight with potentially harmful ultraviolet-B (UV-B) radiation and predation risk, have received recent attention because they may be contributing factors to amphibian population declines occurring on a global scale (Houlahan et al. 2000; Stuart et al. 2004).

Several studies suggest many amphibian population declines are the result of interactions between multiple environmental factors, creating synergistic impacts (Kiesecker et al. 2001; Blaustein and Kiesecker 2002; Bridges and Boone 2003; Collins and Storfer 2003; Hatch and Blaustein 2003; Rohr et al. 2004). As research efforts become increasingly comprehensive in understanding possible synergisms between multiple stressors, it is important to understand individual response strategies. Correlated response, or defense strategies utilizing multiple traits, allows individuals to mediate environmental stress effectively (Arnold and Bennett 1988; Brodie 1989, 1992; Endler 1995). However, studies investigating correlated responses have primarily concen-

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trated on only one stressor. For example, combinations of color pattern and activity rate can function to decrease predation risk in several taxa (Brodie 1992; Forsman and Appelqvist 1998). We posit that correlated trait responses can also resolve conflicts imposed by multiple stressors. Potential conflicts in optimality represent a more realistic picture of complex habitats and may drive organisms to utilize correlated responses to minimize trade-offs.

Amphibian larvae exposed to environmental stressors will generally adopt an optimal response to minimize stress. For many amphibian species, these individual responses involve changes in color and behavior. Color is a highly plastic trait in most amphibian larvae, with intracellular migration of melanin occurring within minutes in response to temperature, predation risk, and ultraviolet (UV) radiation (Duellman and Trueb 1986; Kats and Van Dragt 1986; Garcia and Sih 2003; Garcia et al. 2004). Body color can be a key component of a species' response to stress and influences an individual's ability to communicate, thermoregulate, and avoid predators (Hoppe 1979; Endler 1988). Behavior is also a highly plastic trait in amphibians, changing in response to pressure from predators, UV radiation, and many other factors (Sih 1987; Lima 1998; Kats et al. 2000; Garcia and Sih 2003; Garcia et al. 2004). Color and behavioral responses may interact to form effective defense strategies that mediate single or conflicting environmental stressors, such as predation risk and UV-B exposure.

Skin darkening has been hypothesized to protect amphibians from harmful UV-B exposure and has been observed in several anuran species, including European treefrogs (*Hyla arborea* (L., 1758)), gray treefrogs (*Hyla versicolor* LeConte, 1825), wood frogs (*Rana sylvatica* LeConte, 1825), and African clawed frogs (*Xenopus laevis* (Daudin, 1802)) (reviewed by Blaustein and Belden 2003). Many species of larval salamanders also darken in body color in response to ambient levels of UV-B exposure (Belden and Blaustein 2002a; Garcia et al. 2004), although it is still relatively unclear if this darkening response benefits survivorship in these species. UV-B exposure may also select for simple avoidance behaviors, such as increased activity in an attempt to escape excessive exposure rates, or preference for UV-B protected microhabitats (Nagl and Hofer 1997; Belden et al. 2000; Blaustein et al. 2000; Garcia et al. 2004). Predation is also a strong selective force for amphibian larvae (Sih et al. 1992). Accordingly, larvae show various antipredator responses including changes in both color and behavior. Cryptic coloration, or matching a given background, is a highly effective antipredator strategy (Kats and Van Dragt 1986; Heinen 1994; Endler 1995). Changes in behavior, such as decreased activity and increased refuge use, are also common antipredator responses found in amphibians (e.g., Sih et al. 1992; Werner and Anholt 1993; Hokit and Blaustein 1995; Chivers et al. 1996; Puttlitz et al. 1999).

Some habitats experience increased stress from one environmental factor relative to another; different response strategies may reflect these habitat or species-specific conditions (i.e., certain species may have constrained plasticity in color or behavior, Kats et al. 2000; Garcia et al. 2004). To better understand differences in response across a range of UV-B conditions and predator experience, we tested individuals of two anuran species that inhabit very different elevations. In

general, UV-B intensities become stronger with increasing elevation, exposing species at high elevations to significantly stronger UV-B pressure relative to species at lower elevations (Cockell and Blaustein 2001; Belden and Blaustein 2002b). Individuals with an evolutionary history of high UV-B exposure rates may respond to stressful UV-B exposure rates with a correlated defense strategy geared towards protecting against UV-B damage: increased activity to search for refuge or UV-B protected habitats, and darker skin coloration which may screen out harmful UV-B wavelengths (Fig. 1: strategy UV). Predators may also be more abundant in certain areas. Amphibian populations with a history of high predator-encounter rates may respond with a correlated strategy to defend against intense predation risk: immobility and cryptic body coloration (Fig. 1: strategy CC). We hypothesize that while correlated trait responses mediating a single stressor are highly effective, individual larva exposed to conflicting pressures should respond with strategies designed to defend against both factors by utilizing both color and behavioral changes. In this experiment, this would result in larvae adopting a defense strategy combining immobility to defend against predators with dark skin coloration to screen out UV-B, or the opposite tactic, increased activity to search for refuge with cryptic coloring to reduce conspicuousness to predators (Fig. 1: strategy Both).

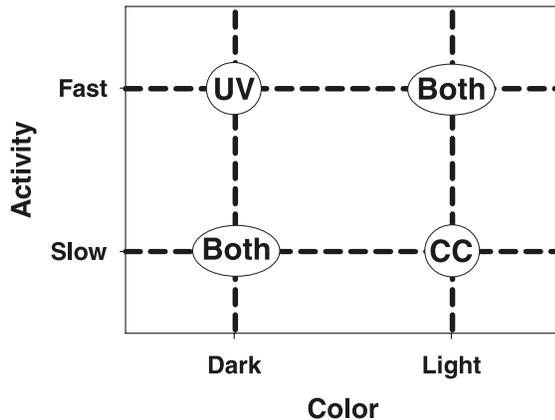
Here, we quantified correlated response strategies to predation risk and UV-B exposure in two species of amphibian larvae: a species inhabiting only low-elevation sites, the red-legged frog (*Rana aurora* Baird and Girard, 1852), and a species found only at relatively high-elevation sites, the cascades frog (*Rana cascadae* Slater, 1939). We addressed the following two questions: (1) do individuals correlate color change and activity rate to reduce UV-B damage and predation risk, and (2) are differences in species response concordant with differences in predator encounter rates and UV-B exposure rates in high-elevation and low-elevation habitats?

System

Rana aurora is restricted to the coastal and low-elevation regions of the Pacific Northwest. In Oregon, the range of *R. aurora* extends from the coastline to the lower elevations (<300 m) of the Oregon Cascade Mountain Range, while the range of *R. cascadae* extends from 1000 to 3000 m elevations in the Oregon Cascade Range. Although *R. aurora* and *R. cascadae* are closely related (Shaffer et al. 2004; Hillis and Wilcox 2005), variation in environmental pressures between habitats have resulted in distinct species differences in characteristics such as breeding phenology (Blaustein et al. 2004), predator-avoidance behaviors (Chivers et al. 1999), and encounter rates with various predators such as rough-skinned newts (*Taricha granulosa* (Skilton, 1849)). For example, the abundance of *T. granulosa* decreases with increasing elevation, thus high-elevation populations of *R. cascadae* have limited encounter rates with *T. granulosa* relative to coastal populations of *R. aurora* (Nussbaum et al. 1983; Blaustein et al. 1995; Hokit and Blaustein 1995).

These two species also vary in their susceptibility to UV-B radiation and natural UV-B exposure rates (Blaustein et al. 1998; Hays et al. 1996). *Rana aurora* only exhibit sublethal effects when exposed to natural levels of UV-B radi-

Fig. 1. Conceptual model representing four correlated color and activity responses to conflicting selection pressures on a light colored substrate. An optimal antipredator response strategy combines decreased activity with cryptic coloration (strategy CC), while an optimal UV-B defense strategy combines dark body coloration with increased activity (strategy UV). Correlated responses to conflicting selection pressures are represented by two distinct strategies (Both) that combine a UV-B defense (dark color vs. increased activity) with an antipredator response (immobility vs. crypsis).



tion; larvae have decreased growth rates as a consequence of UV-B exposure in the embryonic life stage (Blaustein et al. 1996). *Rana cascadae*, however, suffer decreased hatching success when exposed to ambient UV-B radiation (Blaustein et al. 1994), whereas the hatching success of *R. aurora* is not affected by UV-B exposure (Blaustein et al. 1996). UV-B exposure rates in habitats of *R. aurora* and *R. cascadae* differ significantly; larvae from low-elevation populations experience decreased peak UV-B exposure rates (6–8 $\mu\text{W}/\text{cm}^2$) relative to high-elevation populations (14–17 $\mu\text{W}/\text{cm}^2$) (Belden et al. 2000; T.S. Garcia unpublished data; UV-B probe model PMA2100, Solar Light Co., Philadelphia, Pennsylvania).

To explore the question of conflicting environmental pressures and correlated response strategies in these two species, we exposed larvae to both newt chemical cues and two UV-B exposure rates that match levels occurring at high- and low-elevation *Rana* breeding sites. *Rana aurora*, our low-elevation species, has a history of high encounter rates with predatory *T. granulosa* and should respond to predator cues with either an increase in crypsis and (or) a decrease in activity rate. UV-B exposure, however, especially the UV-B treatment replicating high-elevation conditions, should initiate an opposite defense response, (i.e., darker coloration and (or) increased activity). Thus, when exposed to both stressors simultaneously, we expect *R. aurora* to mediate this potential conflict by employing a correlated response strategy that uses both traits to mediate both risks (Fig. 1: strategy Both). We predict that larval *R. cascadae*, the species with the highest potential for UV-B damage, will utilize a correlated response strategy that reduces only UV-B exposure (i.e., dark coloration and increased activity) regardless of the presence of predator chemical cues (Fig. 1: strategy UV). To our knowledge, this is the first empirical study that examines correlated color and behavior response to multiple stressors.

Materials and methods

Rana aurora and *R. cascadae* were collected from breeding sites on dates that corresponded with breeding phenology. Low-elevation larval *R. aurora* were collected from an ephemeral pond complex in Baker Beach State Park, Lane County, Oregon, on 4 May 2005 (elevation ~8 m). High-elevation larval *R. cascadae* were collected from an ephemeral pond near Three Creeks Lake, Deschutes County, Oregon, on 8 July 2005 (elevation ~2000 m). Adult *T. granulosa* were collected from the same sites or adjacent sites at identical elevations. All animals were held at the Oregon State University campus in Corvallis, in a temperature-controlled room with natural photoperiod. All larvae were fed a mixture of rabbit chow and fish flakes (3:1) during the 5–7 day holding period. Fourteen individuals from each species were staged (*R. aurora* = stage 25; *R. cascadae* = stage 25–26 (Gosner 1960)).

We conducted two experiments testing individuals from each species separately. We used a 3 × 2 factorial design with three UV treatments (high, low, absent) and two predation risk treatments (predator chemical cues present and absent). The experiments had five replicates per treatment combination and individuals were randomly assigned to experimental units. Each experimental unit (19 cm × 32 cm clear plastic tub) was placed on a white background (to force the conflict in optimal UV-B and predation-risk-induced color change) and contained one amphibian larva. All experiments were run in a UV-B chamber with constant temperature (16 °C) and photoperiod (12 h light : 12 h dark) equipped with UV-B emitting light bulbs for 6 full days.

Filters were used to manipulate UV-B exposure; Mylar filters excluded 95% of UV-B radiation (control) and high-density polyethylene (HDP) filters removed 15% of UV-B radiation. To achieve high and low UV-B exposure treatments, HDP filters were applied singly (high UV) or doubled up (low UV). UV-B levels were designed to simulate natural conditions at high- and low-elevation breeding sites, yet were below lethal levels (Blaustein et al. 2004). Individuals received a UV-B exposure of $17.18 \pm 0.34 \mu\text{W}/\text{cm}^2$ in the high UV treatment, $5.44 \pm 0.23 \mu\text{W}/\text{cm}^2$ in the low UV treatment, and $0.27 \pm 0.02 \mu\text{W}/\text{cm}^2$ in the UV control treatment.

The UV-B and predation-risk exposure rates were calculated to replicate environmental and diurnal variation. Our lighting regime consisted of a 12 h day but only 5 h of UV-B exposure. In the morning (first 3 h of light) and evening (last 4 h of light), individuals were exposed to only full spectrum bulbs emitting negligible levels of UV-B (Vita-Lite; Durotest Corporation, Fairfield, New Jersey). During peak UV hours (1100–1600) individuals were exposed to UV-B emitting bulbs (UV-313; Q-Panel Inc., Cleveland, Ohio). UV-B was measured with a UV-B probe (model PMA2100; Solar Light Co., Philadelphia, Pennsylvania). Perceived predation risk was generated by the presence of predatory newt chemical cues (*T. granulosa*) from eight adults held for 2 weeks in two 37.5 L tanks and fed conspecific prey ad libitum. Each experimental unit received 1000 mL of dechlorinated water and either 500 mL of dechlorinated water (predation risk absent) or 500 mL newt water (predation risk present). During water changes, the

500 mL of treatment water was added after 500 mL of water was removed from each unit with a glass beaker. Water changes were performed on the 3rd and 5th days of the experiments and larva were fed on the 2nd and 4th days.

Activity rate was quantified by observing each individual for 30 s and summing the number of gridlines each larva crossed during that time period (4 cm square grid under each unit). Individuals were observed every 30 min for a total of 10 observation times during peak UV hours (1100–1600) on the first and last days of the experiment. Color analysis was generated from digital photos taken with a Canon G5 digital camera at the end of the 1st and 6th days of treatment exposure. Images were analyzed using Adobe Photoshop version 7.0. Amphibian larvae vary primarily in brightness (amount of black versus white), with relatively constant chroma and hue (Storfer et al. 1999; Grill and Rush 2000; Garcia and Sih 2003); therefore, all images were converted to gray scale. We quantified brightness with black versus white pixel weights within a selected area on each larva: the body minus the tail region. Higher brightness values indicate a lighter, paler color relative to lower brightness values.

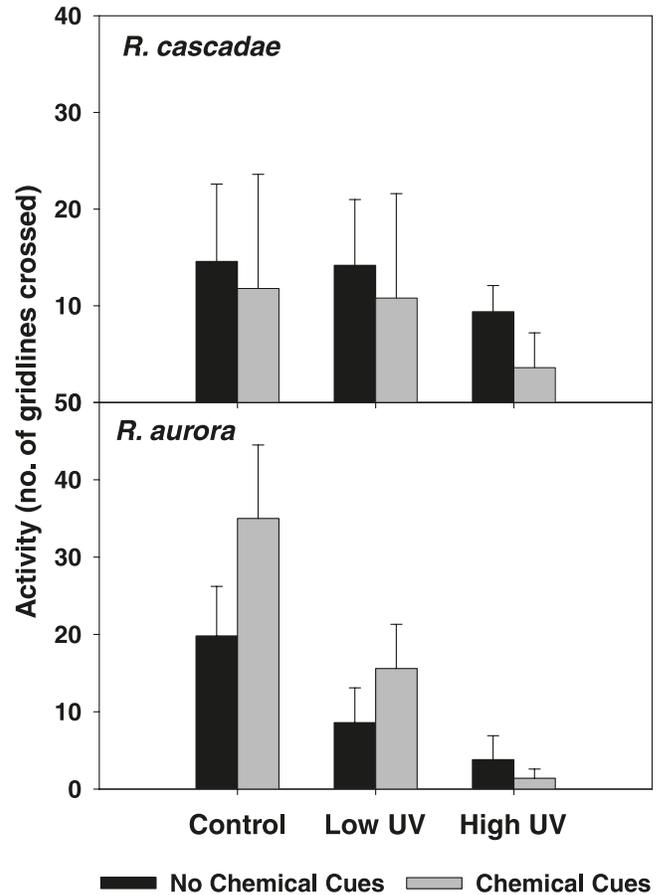
Effects of UV-radiation exposure and newt chemical cues on activity and color over time were analyzed using a repeated-measures multivariate analysis of variance (MANOVA). For activity, our repeated-measures MANOVA analyzed data taken on the 1st and 6th days of the experiment. Only activity data from the 6th day are presented in Fig. 2, as no significant results were found on day 1. For color change, the repeated-measures MANOVA compared the residual data from initial color measurements taken from individuals before treatment exposure and color measurements taken on the 1st and 6th days of treatment exposure (i.e., the degree of color change after 1 day and after 6 days of treatment exposure). Activity and color data are presented in Tables 1 and 2 in a repeated-measures format, with the “between subject” sections describing overall effects of UV-B and predator chemical cues across all days, and the “within subject” sections describing effect of treatments as a function of time. All data were log-transformed to satisfy the conditions of an analysis of variance. In all cases, we used an α of 0.05 as the indicator of statistical significance.

Results

Low-elevation species — *R. aurora*

Larval *R. aurora* significantly decreased their activity rate in response to UV-B exposure (Table 1). This effect changed over time, as larvae significantly decreased activity during the 6 days of exposure to UV-B treatments (Table 1; time \times UV effect). Interestingly, larvae responded to both the low UV treatment and the novel high UV treatment with suppressed activity, rather than the predicted avoidance behavior (i.e., increased activity rate). This suggests that increased activity rates may not be an adequate UV-B defense mechanism for larval *R. aurora*. Low-elevation *R. aurora* also displayed a background-matching response, becoming significantly lighter in body coloration over time (Fig. 3; Table 1). Newt chemical cues did not significantly influence this color change; larvae changed color to better match their

Fig. 2. Activity rates of larval *Rana cascadae* (high-elevation species) and *Rana aurora* (low-elevation species) after 6 days of rough-skinned newt (*Taricha granulosa*) chemical cue and UV-B exposures. Data are means \pm SE. Light bars represent predatory newt chemical cue treatments, whereas dark bars are control treatments. The low UV treatment simulates low-elevation UV-B means ($\sim 6 \mu\text{W}/\text{cm}^2$) and the high UV treatment represents high-elevation UV-B means ($\sim 16 \mu\text{W}/\text{cm}^2$).



light-colored substrate regardless of risk. This default response to match a given background increases crypsis and thereby reduces risk from visual predators.

High-elevation species — *R. cascadae*

Rana cascadae larvae responded to predator chemical cues with a significant decrease in activity rate (i.e., immobility, Table 2). This change in activity took place during the six days of exposure to predator cues (Fig. 2, Time \times CC effect). High elevation *Rana* were also significantly darker in body coloration when both UV-B and predator chemical cues were present (Fig. 3; Table 2). This correlated response was predicted by our conceptual model (Fig. 1) in which larvae respond to a perceived predation threat with decreased activity rates and to UV-B exposure with an increasingly melanistic change in body coloration. In this context, however, *R. cascadae* larvae did not get darker in color unless both stressors were present at UV-B levels matching high elevation conditions (Fig. 3). Additionally, there was a significant Time effect on color (Table 2), with larval color getting lighter over the course of the six day experiment.

Table 1. Repeated-measures MANOVA on UV-B (UV) and rough-skinned newt (*Taricha granulosa*) chemical cue (CC) effects on activity and residual color change of the low-elevation species *Rana aurora*.

Source	Mean square	F	df		P
			Numerator	Denominator	
Activity					
(A) Between subjects					
UV	0.1976	5.139	1	26	0.031*
CC	0.0744	1.936	1	26	0.175
UV × CC	0.0087	0.227	1	26	0.637
(B) Within subjects					
Time	0.1127	2.931	1	26	0.098
Time × UV	0.2006	5.217	1	26	0.031*
Time × CC	0.0001	0.001	1	26	0.987
Time × UV × CC	0.0546	1.420	1	26	0.244
Color change					
(A) Between subjects					
UV	0.0077	0.202	1	26	0.656
CC	0.0407	4.058	1	26	0.313
UV × CC	0.0002	0.005	1	26	0.942
(B) Within subjects					
Time	0.7179	18.66	1	26	0.001*
Time × UV	0.0039	0.101	1	26	0.752
Time × CC	0.0191	0.496	1	26	0.487
Time × UV × CC	0.0807	2.100	1	26	0.159

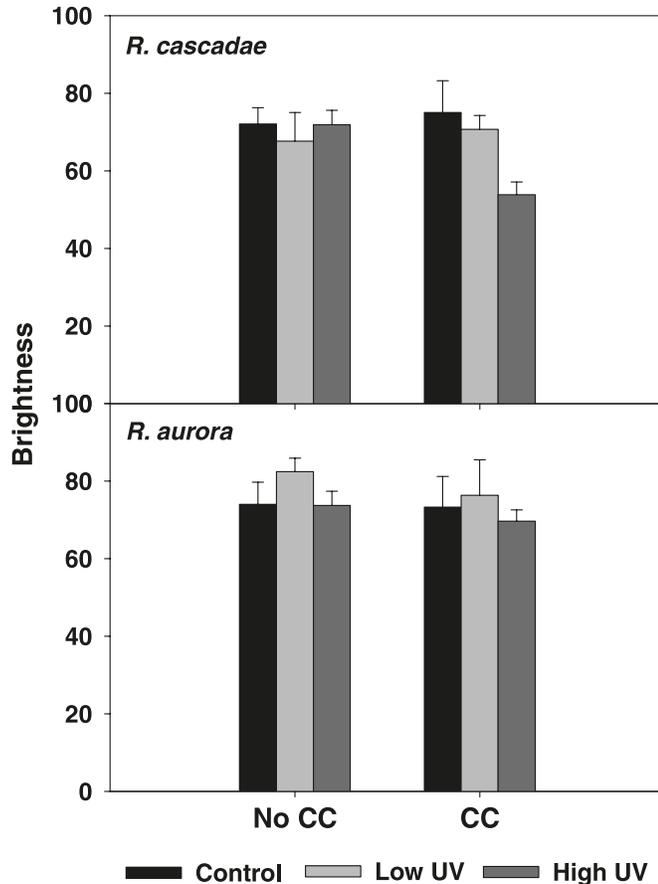
Note: Between-subject effects show differences in overall activity or body color, whereas within-subject effects indicate change over the 6 day exposure period. *, statistically significant values at $P < 0.05$.

Table 2. Repeated-measures MANOVA on UV-B (UV) and rough-skinned newt (*Taricha granulosa*) chemical cue (CC) effects on activity and residual colour change of the high-elevation species *Rana cascadae*.

Source	Mean square	F	df		P
			Numerator	Denominator	
Activity					
(A) Between subjects					
UV	0.0486	1.263	1	26	0.271
CC	0.0119	0.310	1	26	0.582
UV × CC	0.0004	0.012	1	26	0.911
(B) Within subjects					
Time	0.4083	10.61	1	26	0.003*
Time × UV	0.0003	0.008	1	26	0.925
Time × CC	0.2640	6.864	1	26	0.014*
Time × UV × CC	0.0182	0.474	1	26	0.497
Color change					
(A) Between subjects					
UV	0.0140	0.364	1	26	0.551
CC	0.0049	0.128	1	26	0.722
UV × CC	0.2228	5.794	1	26	0.023*
(B) Within subjects					
Time	0.2463	6.405	1	26	0.018*
Time × UV	0.0555	1.444	1	26	0.240
Time × CC	0.0121	0.315	1	26	0.579
Time × UV × CC	0.0054	0.142	1	26	0.708

Note: Between-subject effects show differences in overall activity or body color, whereas within-subject effects indicate change over the 6 day exposure period. *, statistically significant values at $P < 0.05$.

Fig. 3. Color-change data for larval *Rana cascadae* (high-elevation species) and *Rana aurora* (low-elevation species). Higher brightness values indicate a lighter, paler color relative to lower brightness values. Data are means \pm SE from color measurements taken after the 6th day of predatory rough-skinned newt (*Taricha granulosa*) chemical cue (CC) and UV-B treatments.



This overall directional color change was expected in all treatments except High UV, as amphibian larvae tend to background match over time.

Discussion

Rana aurora and *R. cascadae* differed in the strategies individuals used to defend against the combination of predators and UV-B radiation. While both species responded with changes in color and behavior, individuals utilized different response combinations. To reduce detection by potential predators, both species employed effective antipredator responses: *R. aurora* became lighter in color over time, increasing individual crypsis against the light-colored substrate, whereas *R. cascadae* decreased activity and adopted an immobility defense. UV-B exposure resulted in similarly divergent species responses, with larval *R. aurora* decreasing activity rates and *R. cascadae* adopting a darker body color to screen out harmful UV-B wavelengths.

The relative costs and benefits of color change versus behavioral avoidance depend on the ecological context and associated conflicting demands. Predation risk from *T. granulosa* at low elevations is relatively high for anuran larvae, resulting in strong selection for traits reducing over-

all conspicuousness (Nussbaum et al. 1983; Blaustein et al. 1995; Hokit and Blaustein 1995). Larval *R. aurora* became lighter in body color to better match their background regardless of predator presence. Their history of high predation rates may have selected for this default background matching trend, with individuals cryptically blending into their background regardless of predator presence. This color-change defense is beneficial in the presence of predatory newts, but may incur fitness costs in the absence of predation risk (Benard and Fordyce 2003). Potential conflicts between background matching and other factors influencing color include constraints on thermoregulatory response and circadian-rhythm-induced color variation (Binkley et al. 1988; King et al. 1994; Filadelfi et al. 2005). *Rana cascadae* responded to predatory newt chemical cues with decreased activity, a common antipredatory strategy for many amphibian species. Restricted larval activity rates, however, has a high potential cost in terms of reduced feeding and growth rates, ultimately impacting time until metamorphosis (Skelly 1992; Lima 1998). Results from our study suggest that larvae with a history of limited exposure to predators mediate this cost by limiting immobility to times of high predation risk. Conversely, when risk is continuously present, as in low-elevation habitats, larval *Rana* adopt a more chronic antipredator response (i.e., background matching).

Defying prediction, *R. aurora* significantly decreased activity rates after 6 days of exposure to UV-B. We surmise that over the course of 6 days, larval *R. aurora* may have exhausted their search for refuge and decreased activity rates in an attempt to conserve energetic resources. While the combination of cryptic coloration and decreased activity does not fit within the predicted framework of our conceptual model (Fig. 1), we posit that chronic UV-B exposure altered the optimal behavioral response. *Rana cascadae*, however, predictably became darker in body color, but only when exposed to both high UV-B levels and predator cues simultaneously. When only UV-B radiation was present, larvae not only maintained a relatively pale body color, they also showed no change in activity rate in response to UV-B. This lack of response to UV-B alone, again, suggests that larval *Rana* may be utilizing UV-B defense strategies other than color change and activity rate, such as microhabitat preference and (or) physiological adaptations (Blaustein and Belden 2003).

Increased activity and dark coloration may not be the most effective defenses against UV-B. Indeed, Belden and Blaustein (2002a) found that light- and dark-colored larval mole salamanders (genus *Ambystoma* Tschudi, 1838) suffered equally decreased growth rates when exposed to UV-B, and concluded that melanin dispersal did not sufficiently protect larvae from UV-B damage. Changes in activity patterns may also be an insufficient defense against UV-B damage. Alternative UV-B avoidance behaviors include preference for microhabitats with low UV-B exposure (Garcia et al. 2004). Ultraviolet wavelengths attenuate with water depth and dissolved organic carbon concentration (Kirk 1986; Palen et al. 2002). Larvae can reduce UV-B exposure by choosing deeper microhabitats, or spending more time in refugia, thereby limiting the need for color- or activity-based defense strategies.

Species differences in physiological UV-B defenses may best explain our findings. The amount of photolyase activity,

a photo-reactivating enzyme that will repair UV-induced DNA damage, is positively correlated with resistance to UV-B damage (Blaustein et al. 1994). *Rana cascadae* have relatively low levels of photolyase activity compared with other Pacific Northwest anurans and suffer increased mortality as a result of UV-B exposure (Blaustein et al. 1994). *Rana aurora*, however, have relatively high photolyase activity levels compared with other Pacific Northwest amphibians. Blaustein et al. (1996) showed that photolyase levels in *R. aurora* are high in embryos and these levels may be retained in the larvae. Photo-reactivation is an important defense against UV-B damage for many amphibians, and this enzymatic response may allow low-elevation *Rana* to occupy UV-B exposed environments and not respond with behavioral or color defenses. Species differences in photolyase activity may account for the observed lack of predicted response in larval *R. aurora*, while *R. cascadae*, with comparatively lower levels of physiological protection, responded to the combination of high UV-B and predation risk with a predicted color- and behavior-correlated defense strategy.

It is vital to the successful conservation of these two species to better understand how individuals allocate energy in response to multiple environmental stressors. *Rana aurora* and *R. cascadae* have undergone such drastic population declines in the Pacific Northwest that *R. aurora* is listed as a threatened species by the US Fish and Wildlife Service, *R. cascadae* has been a candidate for listing since the early 1990s, and both species are listed as vulnerable by the Oregon Department of Fish and Wildlife (Department of the Interior, US Fish and Wildlife Service in 1991 (Federal Registrar, part 8); Oregon Department of Fish and Wildlife, Wildlife Division, in 1997). There are exceptionally few extant populations of *R. cascadae* in the southern portion of their range in California (Blaustein and Wake 1990; Fellers and Drost 1993) and populations of *R. aurora* have suffered extreme declines in the Willamette Valley, Oregon (see Kiesecker and Blaustein 1998).

As environmental conditions in the Pacific Northwest continue to degrade and interactions between stressors become increasingly complex, conservation efforts will rely on this and similar studies to better understand which combinations of traits are essential in reducing specific impacts on individual fitness. Comparing response strategies between species gives us greater predictive power concerning how stressors may affect amphibian population dynamics. However, our work also suggests that further study is needed to fully realize the range and limitations of correlated trait responses in general, and for amphibians in particular.

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References

Arnold, S.J., and Bennett, A.F. 1988. Behavioral variation in natural populations. V. Morphological correlates of locomotion in

- the garter snake (*Thamnophis radix*). *Biol. J. Linn. Soc.* **34**: 175–190. doi:10.1111/j.1095-8312.1988.tb01955.x.
- Belden, L.K., and Blaustein, A.R. 2002a. UV-B induced skin darkening in larval salamanders does not prevent sublethal effects of exposure on growth. *Copeia*, 2002(3): 748–754. doi:10.1643/0045-8511(2002)002[0748:UBISDI]2.0.CO;2.
- Belden, L.K., and Blaustein, A.R. 2002b. Population differences in sensitivity to UV-B radiation for larval long-toed salamanders. *Ecology*, **83**: 1586–1590.
- Belden, L.K., Wildy, E.L., and Blaustein, A.R. 2000. Growth, survival and behavior of larval long-toed salamanders (*Ambystoma macrodactylum*) exposed to ambient levels of UV-B radiation. *J. Zool. (Lond.)*, **251**: 473–479. doi:10.1111/j.1469-7998.2000.tb00803.x.
- Benard, M.F., and Fordyce, J.A. 2003. Are induced defenses costly? Consequences of predator-induced defenses in western toads, *Bufo boreas*. *Ecology*, **84**(1): 68–78. doi:10.1890/0012-9658(2003)084[0068:AIDCCO]2.0.CO;2.
- Binkley, S., Mosher, K., Rubin, F., and White, B. 1988. *Xenopus* tadpoles melanophores are controlled by dark and light and melatonin without influence of time of day. *J. Pineal Res.* **5**: 87–97. doi:10.1111/j.1600-079X.1988.tb00771.x. PMID:3367263.
- Blaustein, A.R., and Belden, L.K. 2003. Amphibian defenses against ultraviolet-B radiation. *Evol. Dev.* **5**(1): 89–97. doi:10.1046/j.1525-142X.2003.03014.x. PMID:12492415.
- Blaustein, A.R., and Kiesecker, J.M. 2002. Complexity in conservation: lessons from the global decline of amphibian populations. *Ecol. Lett.* **5**: 597–608. doi:10.1046/j.1461-0248.2002.00352.x.
- Blaustein, A.R., and Wake, D.B. 1990. Declining amphibian populations: a global phenomenon? *Trends Ecol. Evol.* **5**: 203–204. doi:10.1016/0169-5347(90)90129-2.
- Blaustein, A.R., Hoffman, P.D., Hokit, D.G., Kiesecker, J.M., Walls, S.C., and Hays, J.B. 1994. UV repair and resistance to solar UV-B in amphibian eggs: a link to population declines? *Proc. Natl. Acad. Sci. U.S.A.* **91**(5): 1791–1795. doi:10.1073/pnas.91.5.1791. PMID:8127883.
- Blaustein, A.R., Beatty, J.J., Olson, D.H., and Storm, R.M. 1995. The biology of amphibians and reptiles in old-growth forests in the Pacific Northwest. U.S. For. Serv. Gen. Tech. Rep. PNW No. GTR 337.
- Blaustein, A.R., Hoffman, P.D., Kiesecker, J.M., and Hays, J.B. 1996. DNA repair activity and resistance to solar UV-B radiation in eggs of the red-legged frog. *Conserv. Biol.* **10**: 1398–1402. doi:10.1046/j.1523-1739.1996.10051398.x.
- Blaustein, A.R., Kiesecker, J.M., Chivers, D.P., Hokit, D.G., Marco, A., Belden, L.K., and Hatch, A. 1998. Effects of ultraviolet radiation on amphibians: field experiments. *Am. Zool.* **38**(6): 799–812.
- Blaustein, A.R., Chivers, D.P., Kats, L.B., and Kiesecker, J.M. 2000. Effects of ultraviolet radiation on locomotion and orientation in roughskin newts (*Taricha granulosa*). *Ethology*, **106**: 227–234. doi:10.1046/j.1439-0310.2000.00519.x.
- Blaustein, A.R., Han, B.A., Fasy, B.A., Romansic, J., Scheessele, E.A., Anthony, R.G., Marco, A., Chivers, D.P., Belden, L.K., Kiesecker, J.M., Garcia, T.S., Lizana, M., and Kats, L.B. 2004. Variable breeding phenology affects the exposure of amphibian embryos to ultraviolet radiation and optical characteristics of natural waters protect amphibians from UV-B in the U.S. Pacific Northwest: a comment. *Ecology*, **85**(6): 1747–1756. doi:10.1890/03-3070.
- Bridges, C.M., and Boone, M.D. 2003. The interactive effects of UV-B and insecticide exposure on tadpole survival, growth and development. *Biol. Conserv.* **113**(1): 49–54. doi:10.1016/S0006-3207(02)00348-8.

- Brodie, E.D. 1989. Genetic correlations between morphology and antipredator behavior in natural populations of the garter snake *Thamnophis ordinoides*. *Nature* (London), **342**: 542–543. doi:10.1038/342542a0. PMID:2586624.
- Brodie, E.D. 1992. Correlational selection for color pattern and antipredator behavior in the garter snake *Thamnophis ordinoides*. *Evolution*, **46**(5): 1284–1298. doi:10.2307/2409937.
- Chivers, D.P., Kiesecker, J.M., Anderson, M.T., Wildy, E.L., and Blaustein, A.R. 1996. Avoidance response of a terrestrial salamander (*Ambystoma macrodactylum*) to chemical alarm cues. *J. Chem. Ecol.* **22**: 1709–1716. doi:10.1007/BF02272409.
- Chivers, D.P., Kiesecker, J.M., Wildy, E.L., Belden, L.K., Kats, L.B., and Blaustein, A.R. 1999. Avoidance response of post-metamorphic anurans to cues of injured conspecifics and predators. *J. Herpetol.* **33**(3): 472–476. doi:10.2307/1565645.
- Cockell, C.S., and Blaustein, A.R. 2001. Ecosystems, evolution and ultraviolet radiation. Springer-Verlag, Berlin.
- Collins, J.P., and Storfer, A. 2003. Global amphibian declines: sorting the hypotheses. *Divers. Distrib.* **9**: 89–98. doi:10.1046/j.1472-4642.2003.00012.x.
- Duellman, W.E., and Trueb, L. 1986. *Biology of amphibians*. The John Hopkins University Press, Baltimore, Md.
- Endler, J.A. 1988. Frequency-dependent crypsis and aposematic coloration. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **319**: 505–523. doi:10.1098/rstb.1988.0062. PMID:2905489.
- Endler, J.A. 1995. Multiple-trait coevolution and environmental gradients in guppies. *Trends Ecol. Evol.* **10**: 22. doi:10.1016/S0169-5347(00)88956-9.
- Fellers, G.M., and Drost, C.A. 1993. Disappearance of the Cascades frog *Rana cascadae* at the southern end of its range, California, USA. *Biol. Conserv.* **65**(22): 177–181.
- Filadelfi, A.M.C., Vieira, A., and Louzada, F.M. 2005. Circadian rhythm of physiological color change in the amphibian *Bufo ictericus* under different photoperiods. *Comp. Biochem. Physiol. A*, **142**: 370–375. doi:10.1016/j.cbpa.2005.09.001.
- Forsman, A., and Appelqvist, S. 1998. Visual predators impose correlational selection on prey color pattern and behavior. *Behav. Ecol.* **9**(4): 409–413. doi:10.1093/beheco/9.4.409.
- Garcia, T.S., and Sih, A. 2003. Color change and color-dependent behavior in response to predation risk in the salamander sister species *Ambystoma barbouri* and *A. texanum*. *Oecologia* (Berl.), **137**: 131–139. doi:10.1007/s00442-003-1314-4.
- Garcia, T.S., Stacy, J., and Sih, A. 2004. Ultraviolet radiation effects on color and behavior in the salamander species *Ambystoma barbouri* and *A. texanum*. *Ecol. Appl.* **14**(4): 1055–1064. doi:10.1890/02-5288.
- Gosner, K.L. 1960. A simplified table for staging anuran embryos and larvae with notes on identification. *Copeia*, 1960: 183–190.
- Grill, C.P., and Rush, V.N. 2000. Analyzing spectral data: comparison and application of two techniques. *J. Linn. Soc.* **69**: 121–138.
- Hatch, A.C., and Blaustein, A.R. 2003. Combined effects of UV-B radiation and nitrate fertilizer on larval amphibians. *Ecol. Appl.* **13**(4): 1083–1093. doi:10.1890/1051-0761(2003)13[1083:CEOURA]2.0.CO;2.
- Hays, J.B., Blaustein, A.R., Kiesecker, J.M., Hoffman, P.D., Pandelova, I., Coyle, D., and Richardson, T. 1996. Developmental responses of amphibians to solar and artificial UVB sources: a comparative study. *Photochem. Photobiol.* **64**(3): 449–456. doi:10.1111/j.1751-1097.1996.tb03090.x. PMID:8806225.
- Heinen, J.T. 1994. The significance of color change in newly metamorphosed American toads (*Bufo a. americanus*). *J. Herpetol.* **28**: 87–93. doi:10.2307/1564685.
- Hillis, D.M., and Wilcox, T.P. 2005. Phylogeny of the New World true frogs (*Rana*). *Mol. Phylogenet. Evol.* **34**: 299–314. doi:10.1016/j.ympev.2004.10.007. PMID:15619443.
- Hokit, D.G., and Blaustein, A.R. 1995. Predator avoidance and alarm-response behavior in kin-discriminating tadpoles (*Rana cascadae*). *Ethology*, **101**: 280–290.
- Hoppe, D.M. 1979. The influence of color on behavioral thermoregulation and hydroregulation. In *Environmental physiology of the amphibians*. Edited by M.E. Feder and W.W. Burggren. Garland Publications Inc., Birmingham, UK. pp. 37–62.
- Houlahan, J.E., Findlay, C.S., Schmidt, B.R., Myer, A.H., and Kuzmin, S.L. 2000. Quantitative evidence for global amphibian population declines. *Nature* (London), **404**: 752–755. doi:10.1038/35008052. PMID:10783886.
- Kats, L.B., and Van Dragt, R.G. 1986. Background color-matching in the spring peeper, *Hyla crucifer*. *Copeia*, 1986: 109–115. doi:10.2307/1444895.
- Kats, L.B., Kiesecker, J.M., Chivers, D.P., and Blaustein, A.R. 2000. Effects of UV-B radiation on antipredator behavior in three species of amphibians. *Ethology*, **106**: 921–931. doi:10.1046/j.1439-0310.2000.00608.x.
- Kiesecker, J.M., and Blaustein, A.R. 1998. Effects of introduced bullfrogs and smallmouth bass on microhabitat use, growth and survival of native red-legged frogs (*Rana aurora*). *Conserv. Biol.* **12**: 776–787. doi:10.1046/j.1523-1739.1998.97125.x.
- Kiesecker, J.M., Blaustein, A.R., and Belden, L.K. 2001. Complex causes of amphibian population declines. *Nature* (London), **410**: 681–684. doi:10.1038/35070552. PMID:11287952.
- King, R.B., Hauff, S., and Phillips, J.B. 1994. Physiological color change in the green treefrog: responses to background brightness and temperature. *Copeia*, 1994(2): 422–432. doi:10.2307/1446990.
- Kirk, J.T. 1986. *Light and photosynthesis in aquatic ecosystems*. Cambridge University Press, Cambridge, UK.
- Lima, S.L. 1998. Stress and decision making under the risk of predation: recent developments from behavioral, reproductive, and ecological perspectives. *Adv. Stud. Behav.* **27**: 215–290. doi:10.1016/S0065-3454(08)60366-6.
- Nagl, A.M., and Hofer, R. 1997. Effects of ultraviolet radiation on early larval stages of the alpine newt, *Triturus alpestris*, under natural and laboratory conditions. *Oecologia* (Berl.), **110**: 514–519. doi:10.1007/s004420050188.
- Nussbaum, R.A., Brodie, E.D., and Storm, R.M. 1983. *Amphibians and reptiles of the Pacific Northwest*. University of Idaho Press, Moscow.
- Palen, W.J., Schindler, D.E., Adams, M.J., Pearl, C.A., Bury, R.B., and Diamond, S.A. 2002. Optical characteristics of natural waters protect amphibians from UV-B in the U.S. Pacific Northwest. *Ecology*, **83**(11): 2951–2957.
- Puttlitz, M.H., Chivers, D.P., Kiesecker, J.M., and Blaustein, A.R. 1999. Threat-sensitive predator avoidance by larval Pacific Treefrogs (Amphibia, Hylidae). *Ethology*, **105**: 449. doi:10.1046/j.1439-0310.1999.00416.x.
- Rohr, J.R., Elskus, A.A., Shepherd, B.S., Crowley, P.H., McCarthy, T.M., Neidzwiecki, J.H., Sager, T., Sih, A., and Palmer, B.D. 2004. Multiple stressors and salamanders: effects of an herbicide, food limitation, and hydroperiod. *Ecol. Appl.* **14**(4): 1028–1040. doi:10.1890/03-5087.
- Shaffer, H.B., Fellers, G.M., Voss, S.R., Oliver, J.C., and Pauly, G.B. 2004. Species boundaries, phylogeography and conservation genetics of the red-legged frog (*Rana aurora draytonii*) complex. *Mol. Ecol.* **13**: 2667–2677. doi:10.1111/j.1365-294X.2004.02285.x. PMID:15315679.
- Sih, A. 1987. Predators and prey lifestyles: an evolutionary and ecological overview. In *Predation: direct and indirect impacts*

- on aquatic communities. *Edited by* W.C. Kerfoot and A. Sih. University Press of New England, Lebanon, N.H. pp. 203–224.
- Sih, A., Kats, L.B., and Moore, R. 1992. Effects of predatory sunfish on the density, drift, and refuge use of stream salamander larvae. *Ecology*, **73**(4): 1418–1430. doi:10.2307/1940687.
- Skelly, D.K. 1992. Field evidence for a cost of behavioral antipredator response in a larval amphibian. *Ecology*, **73**: 704–708. doi:10.2307/1940779.
- Storfer, A., Cross, J., Rush, V., and Caruso, J. 1999. Adaptive coloration and gene flow as a constraint to local adaptation in the streamside salamander, *Ambystoma barbouri*. *Evolution*, **53**(3): 889–898. doi:10.2307/2640729.
- Stuart, S.N., Chanson, J.S., Cox, N.A., Young, B.E., Rodrigues, A.S.L., Fischman, D.L., and Waller, R.W. 2004. Status and trends of amphibian declines and extinctions worldwide. *Science* (Washington, D.C.), **306**: 1783–1786. doi:10.1126/science.1103538. PMID:15486254.
- Werner, E.E., and Anholt, B.R. 1993. Ecological consequences of the trade-off between growth and mortality rates mediated by foraging activity. *Am. Nat.* **142**: 242–272. doi:10.1086/285537.