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Correlated trait responses to multiple selection pressures in larval amphibians reveal conflict avoidance strategies

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ABSTRACT

1. Larval amphibians frequently experience simultaneous, conflicting selection pressures from ultraviolet-B (UV-B) radiation and predation risk, two stressors that can select for opposing defence strategies. When both UV-B and predators are present, individuals can reconcile potential conflicts by correlating particular trait responses (e.g. combining dark or light body colouration with increased or decreased activity rates to create an appropriate multiple stressor strategy). Optimal combinations of body colour and activity rate are predicted to change across an elevation gradient with increasing UV-B exposure.
2. In this study, we tested how larval amphibians combine changes in body colouration and activity rates to create a correlated response to potentially conflicting selection pressures. We quantified activity and colour response in two amphibian species, the pacific treefrog (*Hyla regilla*) and the long-toed salamander (*Ambystoma macrodactylum*), from both high and low elevation populations, and exposed individuals to both a common predator and naturally relevant levels of UV-B.
3. *Hyla regilla* and *A. macrodactylum* individuals from low elevation populations responded with correlated response strategies while high elevation populations did not. Low elevation *H. regilla* coupled decreased activity rates to reduce predator detection with dark body colouration to screen out UV-B. Low elevation *A. macrodactylum* adopted cryptic colouration when predators were present and decreased activity in response to UV-B. Individuals from high elevation *H. regilla* and *A. macrodactylum* populations responded only with changes in activity and not colour change.
4. The observed population differences may reflect variation in selection strengths across an elevation gradient. High elevation habitats may require individuals to focus defence efforts on the greatest potential risk. Additionally, pigmentation changes may not be an adequate defence in these UV-B intense habitats.

Keywords: correlated trait response, defence strategies, multiple stressors, predation risk, ultraviolet radiation

Introduction

A key issue in evolutionary ecology is the effect of conflicting selection pressures on trait response in individuals (Sih, 1987; Lima, 1998). Correlated trait response is one way by which an individual can integrate multiple behavioural, physiological and/or morphological traits to defend against environmental stress. This coordinated defence strategy optimally combines multiple traits and allows individuals to mediate environmental stressors more effectively (Arnold & Bennett, 1988; Brodie, 1989, 1992; Endler, 1995). Studies investigating correlated trait responses have primarily concentrated on only one stressor. For example, combinations of colour pattern and activity rate can function to decrease predation risk in several

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taxa (Brodie, 1992; Forsman & Appelqvist, 1998). Correlated trait responses can also resolve conflicts imposed by multiple stressors by allowing one trait to optimise to one stressor while the coupled trait optimises to the other. These conflicts represent a more realistic picture of complex habitats which may drive organisms to utilise multiple trait responses to minimise trade-offs. Recent studies suggest that widespread declines in many amphibian populations are the result of interactions between multiple environmental factors, creating synergistic impacts and complex associations (Relyea & Mills, 2001; Blaustein & Kiesecker, 2002; Bridges & Boone, 2003; Collins & Storfer, 2003; Hatch & Blaustein, 2003; Rohr *et al.*, 2004).

Amphibian larvae are often exposed to multiple stressors in aquatic habitats, each imposing selection pressure for an optimal response. Many amphibian species respond to a variety of environmental stressors with changes in colour pattern and behaviour. Colour 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 & Trueb, 1986; Kats & Van Dragt, 1986; Garcia & Sih, 2003; Garcia, Stacy & Sih, 2004). Body colour is also a key component of a species' adaptive response to stress and influences an individual's ability to communicate, thermoregulate and avoid predators (Endler, 1988). Behaviour is also highly plastic, changing in response to pressure from predators, UV radiation and many other factors (Lima, 1998; Kats *et al.*, 2000; Garcia & Sih, 2003; Garcia *et al.*, 2004). Colour and behavioural responses may interact to form effective defence strategies that mediate single or conflicting environmental stressors, such as predation risk and UV-B exposure.

Predation is a major source of mortality for amphibian larvae (Wilbur, 1972; Sih, Kats & Moore, 1992), and larvae show various anti-predator adaptations. Cryptic colouration, or matching a given background, is a highly effective anti-predator strategy (Kats & Van Dragt, 1986; Heinen, 1994; Endler, 1995). Changes in behaviour, such as decreased activity and increased refuge use, are also common anti-predator responses found in amphibians (e.g. Sih *et al.*, 1992; Werner & Anholt, 1993; Hokit & Blaustein, 1995; Chivers *et al.*, 1996; Puttlitz *et al.*, 1999). Sublethal levels of UV-B also select for behaviour and colour responses in larval amphibians. UV-B exposure may select for simple avoidance behaviours, such as increased activity in an attempt to escape excessive exposure rates, or preference for refugia and other UV-B protected microhabitats (Nagl & Hofer, 1997; Belden, Wildy & Blaustein, 2000; Blaustein *et al.*, 2000; Garcia *et al.*, 2004). Skin darkening has been hypothesised to protect amphibians from harmful UV-B exposure and has been observed in several anuran species, including *Hyla arborea* (Linnaeus, 1758), *H. versicolor* (LeConte, 1825), *Rana sylvatica* (LeConte, 1825) and *Xenopus laevis* (Daudin, 1802) (reviewed by Blaustein & Belden, 2003). Many species of larval salamanders also darken their body colour in response to increased ambient UV-B exposure (Belden & Blaustein, 2002a; Garcia *et al.*, 2004) although it is still unclear if this darkening response actually increases survivorship in these species.

The relative costs and benefits of colour change versus behavioural avoidance depend on the ecological context and associated conflicting demands. While predation risk may select for cryptic body colouration and decreased activity rates to reduce conspicuousness from predators, UV-B exposure can result in skin darkening and an increase in activity. This conflict can be forced experimentally by placing amphibian larvae on a light coloured background and simultaneously exposing individuals to both stressors. Individuals may correlate colour and behaviour response to mediate both pressures, either by becoming dark coloured (UV-B defense) and immobile (predator defense), or light coloured (cryptic) and highly active (UV-B escape behaviour). This scenario is represented in a conceptual model (Fig. 1) which describes the four possible trait combinations, two of which mediate only one stress (A and D) and two which correlate to defend against damage from both stressors (B and C).

Population differences in preferred response strategies may reflect habitat or species specific conditions; some habitats experience more intense pressure from one stress relative to another, or certain species may have constrained plasticity in colour or behaviour (Kats *et al.*, 2000; Garcia *et al.*, 2004). To better understand differences in response across a range of UV-B and predator experience, we tested individuals of two amphibian species from both high and low elevation populations. In general, UV-B intensities become stronger with increasing elevation, exposing high elevation amphibian populations to significantly stronger UV-B pressure relative to lower populations (Cockell & Blaustein, 2001; Belden & Blaustein, 2002b). We

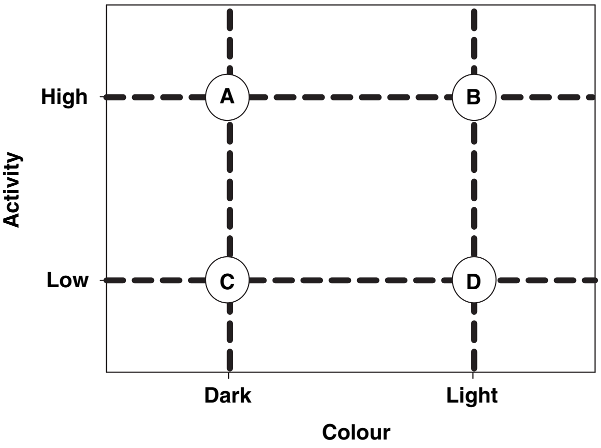

Fig. 1 Conceptual model representing four correlated colour and activity responses to conflicting selection pressures on a light coloured substrate. An optimal anti-predator response strategy (D) combines decreased activity with cryptic colouration, and an optimal UV-B defence strategy (A) combines dark body colouration with increased activity. Correlated responses to conflicting selection pressures are represented by points B and C, combining a UV-B defence (dark colour versus increased activity) with an anti-predator response (immobility versus crypsis), creating two distinct strategies.

predicted a stronger UV-B defense response in individuals from high elevation populations (Fig. 1, strategy A) compared with those from lower elevations. Predators may also be more abundant in certain areas, thus amphibian populations with a history of high predator encounter rates may respond with strategy B.

In this study, we quantified correlated trait responses to predation risk and UV-B exposure in two species of amphibian larvae: the pacific treefrog [*Hyla regilla* (Baird & Girard, 1852)] and the long-toed salamander [*Ambystoma macrodactylum* (Russell and Anderson, 1956)]. We addressed two specific issues: (i) how individuals correlate colour and behaviour responses to mediate conflicting selection pressures and (ii) if population and species differences in response are concordant with habitat differences in predator encounter rates and UV-B exposure. We predicted that both species would correlate activity rates with colour change to defend against simultaneous exposure to UV-B and predation risk, and that preferred strategies for each species would differ across a stress gradient.

Methods

Hyla regilla (hereafter *Hyla*) is widespread across western North America, ranging from Baja California to British Columbia. In Oregon, *Hyla*'s range extends from the coastline to the upper elevations (*c.* 2000 m) of the Oregon Cascade Mountain Range. *Ambystoma macrodactylum* (hereafter *Ambystoma*) is also common in Oregon with a range from the Willamette Valley to the high Oregon Cascades (*c.* 2000 m). High and low elevation populations of both species breed in ephemeral ponds and experience varying degrees of predation from the roughskin newt [*Taricha granulosa* (Skilton, 1849)], predatory insects and other amphibians. Since the abundance of roughskin newts decreases with increasing elevation, high elevation amphibian prey populations have fewer encounters with newts than valley or coastal amphibian populations (Nussbaum, Brodie & Storm,1983; Blaustein *et al.*,1995; Hokit & Blaustein, 1995). In addition, larvae from low elevation populations experience less intense peak UV-B exposure rates (6–8 μW cm^{-2}) relative to high elevation populations (14–17 μW cm^{-2}) (Belden *et al.*, 2000; T. S. Garcia, unpubl. data, UV-B probe, model PMA2100; Solar Light Co., Philadelphia, PA, U.S.A.).

While high and low populations differ in newt encounter rates and UV-B exposure, these two species were also chosen because they vary in their susceptibility to UV-B radiation (Blaustein *et al.*, 1998). *Hyla* are relatively resistant to UV-B damage; hatching success and larval survivorship are not impacted by UV-B exposure (Ovaska, Davis & Novales Flamerique, 1997; Blaustein *et al.*, 1998). *Ambystoma* are more susceptible to UV-B damage in the embryo and larval stages, experiencing decreased hatching success, growth and development rates (Blaustein *et al.*, 1997; Belden *et al.*, 2000; Belden & Blaustein, 2002a). Moreover, *Ambystoma* individuals from low elevation populations are more susceptible to damage from naturally relevant levels of UV-B than individuals from high elevation populations (Belden & Blaustein, 2002b).

Hyla regilla and *A. macrodactylum* were collected from low and high elevation sites at dates which corresponded with breeding phenology. Low elevation *Hyla* larvae were collected from four ephemeral ponds (elevation *c.* 8 m) in Baker (Lane Co., Beach State Park, OR, U.S.A.) on 4 May 2004. Low elevation *Ambystoma* larvae were collected from a 5-acre flooded woodland (elevation *c.* 75 m) 15 km southeast of Corvallis Oregon, on 27 April 2004. High elevation *Hyla* and *Ambystoma* larvae were collected from a complex of ephemeral ponds (elevation *c.* 2000 m) near Deschutes Co. (Three Creeks Lake, OR, U.S.A.) on 8 July and 19 August respectively. *Taricha granulosa* adults were collected from the same sites or adjacent sites at identical elevations. All animals were held in 37.8 L glass tanks at densities of 20 individuals per tank at the Oregon State University campus in Corvallis in a temperature controlled room with natural photoperiod (16 °C, 14 L : 10 D). Anuran larvae were fed a mix of rabbit chow and fish flakes (3 : 1) and salamander larvae were fed Daphnia, *ad libitum* during the 5–7 day holding period. Fourteen individuals from each population were measured and/or staged at the end of the holding period (*Ambystoma* = 22.0 mm ± 0.3 total length, *Hyla* = Gosner stage 25–26; Gosner, 1960).

We conducted four experiments testing members from each site separately. We used a 3 × 2 factorial design; three UV-B treatments, (high, low and absent) and two predation risk treatments [predator chemical cues (CC) present and absent]. The experiment had five replicates per treatment combination and individuals were randomly assigned to experimental units. Each experimental unit (19 × 32 cm clear plastic tubs) was placed on a white background and contained one amphibian larva. All experiments were run in a UV-B chamber with constant temperature and photoperiod (16º°C, 14 L : 10 D) equipped with UV-B emitting light bulbs for full 6 days.

Filters were used to manipulate UV-B exposure; Mylar filters excluded 95% of UV-B radiation (UV absent) and high density polyethylene filters removed 15% of UV-B radiation (UV present). To achieve high and low UV-B exposure treatments that simulated realistic conditions at high and low elevations, high density polyethylene filters were applied singly (high elevation) or doubled-up (low elevation). Exposures were carefully designed so that amphibians received UV-B exposure relevant to their natural histories yet below lethal levels (Blaustein *et al.*, 2004). Individuals received an exposure rate of 16.7 ± 0.16 μW cm^{-2} in the high UV-B treatment, 5.68 ± 0.32 μW cm^{-2} in the low UV treatment and 0.24 ± 0.01 μW cm^{-2} in the UV-B absent treatment. Water temperature across units remained consistent regardless of UV-B exposure rates. To simulate a natural UV-B environment, our lighting regime consisted of a 14-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-Life; Durotest Corporation, Fairfield, NJ, U.S.A.). During peak UV hours (11:00–16:00 hours) individuals were exposed to UV-B emitting bulbs (UV-313; Q-Panel Inc., Cleveland, OH, U.S.A.). UV-B was measured with a UV-B probe (model PMA2100; Solar Light Co., Philadelphia, PA, U.S.A.).

Perceived predation risk was generated by the presence of predatory newt (*T. granulosa*) CC from eight adults held for 1 week in two 37.5-L tanks and fed *ad libitum* (prey items were conspecifics of the population being tested at the time). Each experimental unit received either 1500-mL of de-chlorinated water (predation risk absent) or 1000 mL of de-chlorinated water and 500 mL newt water (predation risk present). Water changes where 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^2 grid under each unit). Individuals were observed every 30 min for a total of 10 observation times during peak UV hours (11:00–16:00 hours) on the first and last days of the experiment. Colour analysis was generated from digital photos taken with a Canon G5 digital camera at the end of the 1st and 6th day of treatment exposure. Images were analysed using Adobe Photoshop 7.0 (Adobe Systems Inc., San Jose, CA, U.S.A.). Since amphibian larvae vary primarily in brightness values (amount of black versus white), with relatively constant chroma and hue values (Storfer *et al.*, 1999; Grill & Rush, 2000; Garcia & Sih, 2003), all images were converted to greyscale. We quantified brightness with black versus white pixel weights within a selected dorsal region on each larva: the body minus the tail region in *Hyla*, and the head area up to the beginning of the external gills in *Ambystoma*. Brightness values were less impacted by internal structures in these regions. Larger brightness values indicate a lighter, paler colour relative to smaller brightness values.

Effects of UV-B radiation exposure and newt CC on activity and colour over time were analysed using repeated measures analysis of variance (ANOVA). For activity, our repeated measures ANOVA analysed data taken on the 1st and 6th days of the experiment. Only activity data from the 6th day are presented, as no significant results were found on day 1. For colour, the repeated measures ANOVA used data taken on the 1st and 6th days of the experiment with initial colour (i.e.

data taken from individuals before treatment exposure) as a covariate. Activity and colour data are presented in a repeated measures format, with the 'between subject' section describing overall effects of treatments across all days, and the 'within subject' section describing effect of treatments as a function of time (i.e. days 1 and 6). All data were log-transformed in order to satisfy the conditions of ANOVA. In all cases we used $P < 0.05$ as the indicator of statistical significance and we used one-tailed criteria for tests where we had clear *a priori* hypotheses.

Results

Low elevation populations

Hyla regilla. Low elevation *Hyla* larvae significantly decreased their activity rate in response to predatory newt CC (Table 1, CC effect: $P = 0.006$). Activity changed over time (Table 1, time effect: $P = 0.004$) however, as larvae significantly decreased activity over the 6-day exposure period to CC (Table 1, time × CC effect: $P = 0.038$). Low elevation *Hyla* were also significantly darker in body colouration in response to UV-B exposure (Fig. 2; Table 1, UV effect: $P = 0.045$). These results were predicted by strategy C in our conceptual model (Fig. 1), in which larvae respond to a perceived predation threat with decreased activity rates and to UV-B exposure with a change in body colouration. In addition, larvae decreased activity in the high UV-B exposure treatment over the 6 days of exposure (Table 1, time × UV interaction: $P = 0.038$). Larvae responded to this novel UV-B exposure rate with suppressed activity, rather than the predicted avoidance behaviour (i.e. increased activity rate).

Ambystoma macrodactylum. Low elevation *Ambystoma* larvae changed colour when newt cues were present, becoming significantly lighter and more cryptically coloured (Fig. 2; Table 2, CC effect: $P = 0.047$). In addition, *Ambystoma* larvae decreased activity rates in response to UV-B exposure during the 6 days of

Table 1 Repeated measures ANOVA on *Hyla regilla* larvae

	H. regilla – low elevation				*H. regilla* – high elevation			
Source	d.f.	MS	*F*	*P*-value	d.f.	MS	*F*	*P*-value
Activity								
Between subjects								
UV	2	0.517	0.489	0.620	2	0.535	1.002	0.382
CC	1	9.652	9.117	0.006*	1	8.489	15.89	0.001*
UV × CC	2	0.134	0.127	0.882	2	0.885	1.656	0.212
Error	24	1.059			20	0.534		
Within subjects								
Time	1	5.432	10.35	0.004*	1	1.504	6.882	0.015*
Time × UV	2	1.967	3.749	0.038*	2	0.233	1.066	0.360
Time × CC	1	2.535	4.831	0.038*	1	4.895	22.39	0.001*
Time × UV × CC	2	0.536	1.022	0.375	2	0.179	0.821	0.452
Error	24	0.525			20	0.219		
Colour change								
Between subjects								
UV	2	422.59	3.587	0.045*	2	58.210	0.181	0.836
CC	1	167.44	1.421	0.246	1	25.114	0.078	0.782
UV × CC	2	233.61	1.983	0.162	2	38.270	0.119	0.888
Initial colour	1	3897.5	33.08	0.001*	1	1153.6	3.585	0.071
Error	22	117.83			23	321.77		
Within subjects								
Time	1	25.578	0.188	0.668	1	814.57	11.86	0.002*
Time × UV	2	179.85	1.325	0.286	2	225.47	3.283	0.056
Time × CC	1	13.953	0.103	0.751	1	45.812	0.667	0.422
Time × UV × CC	2	189.12	1.394	0.269	2	77.536	1.129	0.341
Time × initial	1	17.811	0.131	0.721	1	990.64	14.42	0.001*
Error	22	135.69			23	68.672		

UV, ultraviolet; CC, chemical cues.
*statistically significant values at $P < 0.05$.

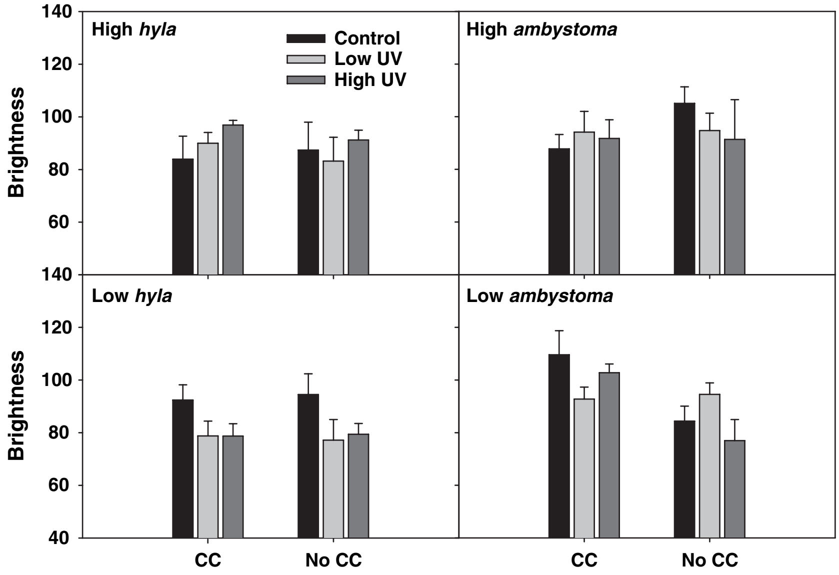

Fig. 2 Colour change data for high elevation (upper panels) and low elevation (lower panels) larval *Hyla regilla* and *Ambystoma macrodactylum*. Larger brightness values indicate a lighter, paler colour relative to smaller brightness values. Data are means ± SE from colour measurements taken after the 6th day of predatory newt chemical cue (CC) and UV-B treatments.

Table 2 Repeated measures ANOVA on *Ambystoma macrodactylum* larvae

	A. macrodactylum – low elevation				*A. macrodactylum* – high elevation			
Source	d.f.	MS	*F*	*P*-value	d.f.	MS	*F*	*P*-value
Activity								
Between subjects								
UV	2	0.873	0.661	0.528	2	0.470	0.933	0.409
CC	1	0.912	0.691	0.416	1	0.442	0.877	0.360
UV × CC	2	0.222	0.168	0.846	2	1.511	2.997	0.072
Error	19	1.320			21	0.504		
Within subjects								
Time	1	44.97	109.4	0.001*	1	23.66	78.45	0.001*
Time × UV	2	3.885	9.455	0.001*	2	1.172	3.887	0.037*
Time × CC	1	0.114	0.277	0.605	1	0.328	1.087	0.309
Time × UV × CC	2	0.166	0.404	0.673	2	0.233	0.772	0.475
Error	19	0.411			21	0.302		
Colour change								
Between subjects								
UV	2	123.87	0.529	0.598	2	203.9	0.861	0.439
CC	1	726.17	3.103	0.047*	1	21.52	0.091	0.766
UV × CC	2	112.35	0.480	0.626	2	196.1	0.828	0.452
Initial colour	1	1274.5	5.446	0.031*	1	4126.8	17.42	0.001*
Error	18	234.01			19	236.9		
Within subjects								
Time	1	40.69	0.304	0.588	1	397.2	4.921	0.039*
Time × UV	2	19.33	0.145	0.866	2	57.53	0.713	0.503
Time × CC	1	50.53	0.378	0.546	1	45.44	0.563	0.462
Time × UV × CC	2	59.92	0.448	0.646	2	59.06	0.732	0.494
Time × initial	1	28.61	0.214	0.649	1	276.1	3.420	0.080
Error	18	133.7			19	80.73		

UV, ultraviolet; CC, chemical cues.

exposure (Table 2, time × UV interaction: $P = 0.001$). Our conceptual model couples cryptic colouration with a decrease in activity (strategy D, Fig. 1) only when both traits are used to decrease conspicuousness to predators. The decrease in activity rate in this study can be directly attributed to UV-B exposure and not predation risk, which suggests that predicted strategies B and C (Fig. 1) may not be the only options available to larva experiencing both selection pressures simultaneously.

High elevation populations

Hyla regilla. *Hyla* larvae responded to predator CC with a significant decrease in activity rate (Table 1, CC effect: $P = 0.001$). Larval activity changed significantly over time (Table 1, time effect: $P = 0.015$), decreasing during the 6 days of treatment exposure (Fig. 3). High elevation *Hyla* larvae did not respond to either UV-B or predator CC with a change in body colouration (Fig. 2; Table 1). There was a marginally significant effect of UV-B on colour ($P = 0.056$), but this result may be a function of larval colour getting darker across treatments over time (Table 1, time effect: $P = 0.002$). This overall directional colour change was unexpected however, as amphibian larvae on light backgrounds tend to match their backgrounds over time.

Ambystoma macrodactylum. High elevation *Ambystoma* larvae decreased activity in response to UV-B (Table 2, time × UV interaction: $P = 0.037$). Similar to the low elevation population, this change in activity occurred during the 6 days of treatment exposure (Table 2, time effect: $P = 0.001$). There was a marginally significant effect of predator CC on activity (Fig. 3; Table 2, UV × CC interaction: $P = 0.072$), but this response to newts was apparently overwhelmed by the presence of UV-B (Fig. 3). Larvae did not change colour in response to either stress, but did get lighter over time (Table 2, time effect: $P = 0.039$), presumably in response to the light coloured background (Fig. 2).

Discussion

Individuals from low and high elevation habitats differed in the strategies used to defend against predation risk and UV-B damage. When exposed to UV-B, low elevation *Hyla* decreased activity in response to predator CC and darkened body colour, whereas low elevation *Ambystoma* became lighter in colour to match their background cryptically and reduce predation risk, and decreased activity rates when exposed to UV-B. Individuals from high elevation populations did not exhibit the correlated responses found in the low elevation individuals. High elevation *Hyla* decreased activity rates in response to predator CC, but did not respond to either stressor with colour change. Likewise, high elevation *Ambystoma* decreased activity in response to UV-B, but did not alter body colour when exposed to either stressor.

Larvae from low elevation *Hyla* and *Ambystoma* populations changed both body colour and activity rate in a correlated defence response, albeit using different trait combinations. Low elevation *Hyla*

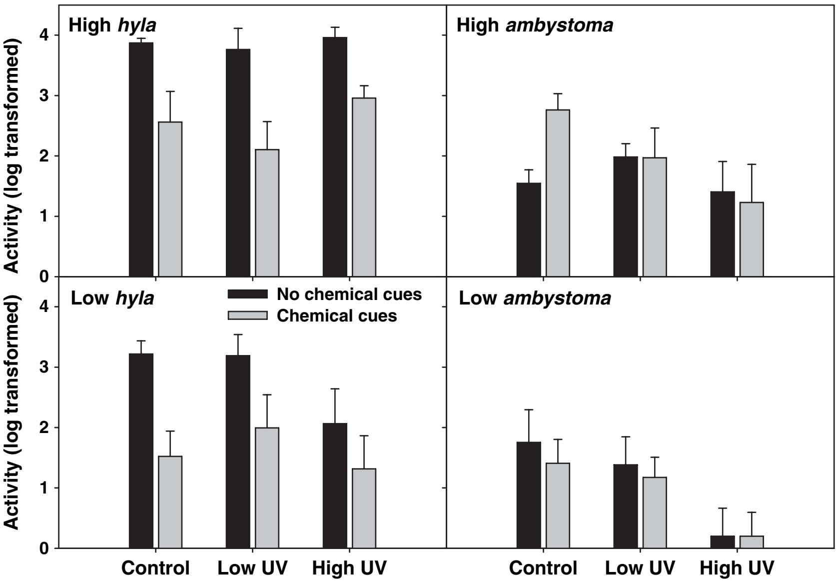

Fig. 3 High elevation (upper panels) and low elevation (lower panels) larval *Hyla regilla* and *Ambystoma macrodactylum* activity rates after 6 days of newt chemical cue (CC) and UV-B treatment exposure. Data are means + SE. Light bars represent predatory newt CC treatments, dark bars are control treatments. The low UV treatment simulates low elevation UV-B means, *c.* 6 μW cm^{-2}, and the high UV treatment represents high elevation UV-B means, *c.* 16 μW cm^{-2}.

larvae employed a combination of responses (strategy C, Fig. 1), we predicted would decrease conspicuousness to predators with behaviour, and reduce UV-B damage with a protective melanistic barrier within the integument. Low elevation *Ambystoma* larvae also correlated colour and activity responses by adopting cryptic body colouration and decreasing activity rate in the presence of UV-B. This combination of cryptic body colouration and a decrease in activity rate was originally described as an effective anti-predator combination (strategy B, Fig. 1) with little protective value in UV-B exposed environments. We predicted that exposure to UV-B would elicit an escape response (i.e. increased activity) with the aim of quickly finding UV-B protected microhabitats. We surmise that during the 6 days of exposure to UV-B, *Ambystoma* larvae may have exhausted their search for refugia and then decreased activity rates in an attempt to conserve energetic resources. While the combination of cryptic colouration and decreased activity does not fit within the predicted framework of our conceptual model, we posit that chronic UV-B exposure altered the optimal behavioural response.

Individuals from our high elevation populations did not respond with correlated colour and activity patterns. *Hyla* decreased activity in the presence of predator CC, similar to the anti-predator response in low elevation *Hyla*. However, high elevation *Hyla* did not change colour in response to either UV-B or predation risk. Similarly, high elevation *Ambystoma* showed no significant change in body colour in response to either stress. This is a surprising result, as these populations inhabit ponds with relatively high levels of UV-B. High elevation *Ambystoma* decreased activity in the presence of UV-B exposure, but it is unlikely that this is an effective defence against UV-B damage. More likely, the reduction in activity rates over time, as seen in both high and low elevation *Ambystoma*, was a response to chronic exposure. These results suggest that individuals from high elevation populations may be employing alternate defenses against UV-B damage.

These results show a conflict in optimal colour response when exposed to both predator CC and UV-B in low elevation populations of *Hyla* and *Ambystoma*. Larvae were unable to adopt an appropriate body colouration for both selection pressures. Cryptic background matching in response to predators required larvae to lighten body colour, while UV-B forced a darkening response in both species. This conflict was not present, however, in larval activity response. We predicted that UV-B exposure would select for high activity rates, while immobility would be the chosen anti-predation strategy. This conflict could then be mediated by employing colour change as a defence against UV-B damage or predators. However, low elevation *Ambystoma* decreased their activity rates in response to UV-B exposure, Again, we posit this is a result of chronic exposure and an attempt to conserve energetic resources.

Adopting a darker body colour my not be sufficient protection against damage from high rates of UV-B exposure. While many amphibian species darken body colour in response to UV-B (Belden & Blaustein, 2002a; Blaustein & Belden, 2003; Garcia *et al*., 2004), no studies have linked increased survivorship with darker integuments in UV-B exposed environments. Indeed, Belden & Blaustein (2002a) found that light and dark coloured *Ambystoma* larvae suffered equally decreased growth rates when exposed to UV-B. Our high elevation results provide further evidence that skin darkening may not be the most effective defence against UV-B in high elevation habitats. We found that individuals from habitats with comparatively high UV-B exposure showed limited colour response.

Individuals at high elevations, with an evolutionary history of intense UV-B exposure, may be utilising alternative UV-B defence mechanisms, such as physiological adaptations and microhabitat preference (Blaustein & Belden, 2003). *Hyla* have relatively high photoreactivating enzyme (photolyase) activity that repairs UV-induced DNA damage. The amount of photolyase activity is positively correlated with resistance to UV-B damage (Blaustein *et al*., 1994). While photolyase activity has not been quantified in *Hyla* populations across an elevation gradient, high UV-B environments may be selecting for increased repair activity. Population differences in enzyme activity could account for our observed population differences in colour and behaviour response.

Preference for microhabitats with low UV-B exposure is another mechanism by which larvae can protect themselves against UV-B damage (Garcia *et al*., 2004). UV wavelengths attenuate with water depth and dissolved organic carbon concentration (Kirk, 1986; Palen *et al*., 2002). Larvae at high elevations can reduce UV-B exposure by choosing deeper microhabitats, or spending more time in refugia, thereby limiting the need for colour or activity based defence strategies. There is evidence for population differences in *Ambystoma* microhabitat choice: high elevation females oviposit their eggs underneath rocks, while females from low elevation populations attach egg clutches to UV-B exposed vegetation (Garcia, pers. obs.). This oviposition behaviour demonstrated by females from high elevation populations protects eggs from UV-B and may have evolved, at least in part, as an adaptation against the harmful effects of UV-B exposure (Kiesecker, Blaustein & Belden, 2001; Marco *et al*., 2001). This population difference in behaviour may also be indicative of additional microhabitat preferences in high elevation populations that protect against UV-B damage.

Individuals of both species from high elevation populations changed skin colour over time, regardless of treatment, but in opposite directions (Tables 1 & 2). Background colour is a strong factor in controlling amphibian colouration (Bagnara & Hadley, 1973; Garcia & Sih, 2003) and high elevation *Ambystoma* larvae became lighter in overall body colour after 6 days on a white background. High elevation *Hyla* larvae, however, became darker in overall body colour over time; a response that cannot be attributed to predation risk, UV-B exposure or background colour. The selection pressures used in this study, newt CC and UV-B radiation, may not be responsible for selecting body colour in high elevation populations of *Hyla*, and the release from environmental pressures not tested in this study may be contributing to this gradual colour change. Additionally, many species of amphibian will darken in body colouration in response to cold temperatures (Hoppe, 1979; King, Hauff & Phillips, 1994; Garcia, Straus & Sih, 2003); the restriction of thermoregulatory options in our experimental set-up may have precipitated *Hyla* larvae to change colour in an attempt at behavioural thermoregulation.

Interestingly, high elevation *Ambystoma* larvae increased activity rates in the presence of predatory newt CC (Fig. 3). Amphibian larvae typically do the opposite and become immobile when exposed to predators (Sih, 1987; Hokit & Blaustein, 1995; Lima, 1998). High elevation *Ambystoma* may have employed this particular escape response because of a history of low encounter rates and inexperience with this species of predator. Or, in specific habitats and communities, an increase in activity may be the appropriate anti-predator behaviour (Urban, 2007). Regardless, this increase in activity was not statistically significant and appears to have been overwhelmed by a UV-B-induced activity response, and disappears in the presence of both low and high levels of UV-B radiation.

Another interesting deviation from the predicted correlated response strategies occurred in low elevation *Hyla* individuals when exposed to high levels of UV-B. We designed this experiment to test colour and behaviour responses to UV-B levels that simulate conditions at both low and high elevations. As a result, individuals from low elevation populations were subjected to *c.* 2–3× the UV-B exposure rate typically experienced in their natural habitats (Belden *et al*., 2000; Garcia, pers. obs.). We predicted this dramatic increase in UV-B intensity would force larvae to modify both colour and behaviour to defend against UV-B damage. The direction of response was not as we predicted; larvae significantly decreased activity rate in the presence of high UV-B levels (Fig. 2). Nevertheless, this change in activity rate implies that these individuals are highly plastic in their ability to respond to changing environmental conditions. This behavioural switch in response to UV-B exposure rates helps us better understand how amphibians may respond with correlated response strategies as environmental conditions continue to degrade.

Our study illustrates some of the defence strategies that can be used by amphibian larvae in response to conflicting selection pressures. The conservation of amphibians has become an increasingly difficult and important issue. We observed significant differences across species and populations in larval responses to multiple stressors. Comparing response strategies across populations and between species gives greater power to predict how stressors may affect amphibian population dynamics. As the environment changes due to climate change, contamination and other pressures, it is essential to better understand how individuals correlate trait responses to such changes with defence strategies. Our work suggests that further study is needed to fully appreciate the range and limitations of correlated trait responses in general, and for amphibians in particular.

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