

## A tad too high: Sensitivity to UV-B radiation may limit invasion potential of American bullfrogs (*Lithobates catesbeianus*) in the Pacific Northwest invasion range

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### Abstract

Biological invasion potential can be strongly influenced by abiotic factors such as temperature, water availability, and solar radiation. Invasive species that possess phenotypically plastic traits can mediate impacts from these stressors, but may be unable to recognize and respond to dangerous levels in a novel environment. Understanding potential constraints on appropriate trait responses induced by abiotic stressors can aid in the management and control of important invaders. Our study explored tolerance and plastic trait response to UV-B radiation in an invasive anuran, the American bullfrog (*Lithobates catesbeianus* Shaw, 1802). We experimentally quantified larval mortality rates and color change responses across two larval size classes. In a second experiment, we investigated the potential for a correlated color change and behavioral (refuge use) response in the small size class. We predicted that individuals would respond to stressful and potentially harmful UV-B exposure rates with darkening of body coloration, and when refuge was available, a correlated defense strategy utilizing both color change and refuge. We found an increase in larval mortality across both size classes at UV-B exposure rates typical to both low and high elevation aquatic breeding sites (10–12  $\mu\text{W}/\text{cm}^2$  and 20–24  $\mu\text{W}/\text{cm}^2$ , respectively). Only bullfrog larvae in the small size class exhibited a darkening in body color when exposed to high UV-B treatments. Although this smaller size class did exhibit color plasticity, individuals did not correlate changes in body coloration with changes in refuge use. These results suggest ontogenetic differences (estimated by size class) in plastic color response to UV-B stress as well as constraints on behavioral use of refuge. These findings are important in understanding differences in bullfrog occupancy of breeding habitats across an elevational gradient, particularly in Oregon's Cascade Mountain Range, where bullfrog distributions are currently limited at elevations above 1000m.

**Key words:** American bullfrog, invasion, ultraviolet radiation, amphibian, color, behavior, plasticity

### Introduction

Biological invasion potential is influenced by a suite of factors including introduction pathway, biotic resistance, and tolerance to abiotic stressors (Blackburn et al. 2011; Souza et al. 2011). The ability to cope with a wide range of environmental conditions can be a primary determinant of non-native species persistence in novel habitats. Invading populations may be inherently constrained by abiotic tolerances; further, source populations may differ in evolutionary potential, producing divergent invasion dynamics (Gilchris and Lee 2007). As such, successful colonization may vary across spatiotemporal stress gradients within the introduced range (Chase et al. 2003;

Leibold 1998). However, the influence of constrained trait response on invasion failure is largely unknown since unsuccessful introductions are rarely studied experimentally (Simberloff and Gibbons 2004; Zenni and Nunez 2013).

Abiotic stressors, including biologically harmful ultraviolet radiation (UV-B, 280–315 nm), can be important regulators of biological invasions and vary across ecological and elevational gradients (Hayes and Barry 2008). UV-B often has strong impacts on terrestrial and aquatic organisms (Caldwell et al. 1998; Hader 2000), affecting survivorship, growth, and behavior across many trophic groups and life stages (Bancroft et al. 2007; Thomas et al. 2012). Functionally significant trait responses to UV-B

exposure that enhance tolerance or mediate damage include behavioral avoidance, protective morphological characteristics, and physiological DNA-repair mechanisms (Blaustein et al. 1994; Garcia et al. 2004; Salih et al. 2000). Rapid selection for these trait responses can be limited in invasive populations due to small numbers of initial colonizers and reduced genetic variation (Lee 2002). Therefore, phenotypic plasticity may be the primary mechanism that allows invasive individuals to respond appropriately to harmful UV-B conditions.

Phenotypically plastic trait response can be a highly successful strategy for avoiding damage from UV-B exposure (Peacor et al. 2006). Many amphibian species are particularly sensitive to UV-B (Bancroft et al. 2008; Searle et al. 2010) and may be capable of avoiding UV-B damage via several plastic mechanisms, including skin darkening and behavioral preference for protected microhabitats (Garcia et al. 2004; Gevertz et al. 2012). Skin darkening occurs rapidly via a hormone-regulated, readily-reversible process when light-absorbing melanin migrates to obscure reflective pigment cells (Bagnara et al. 1968; Kats and Van Dragt 1986; Rudh and Qvarnström 2013; Sköld et al. 2012). Although melanism as a photoprotective response in amphibians needs to be explored further (Belden and Blaustein 2002; Rudh and Qvarnstrom 2013), it is hypothesized to be a defensive response to UV-B exposure (Cockell and Blaustein 2001).

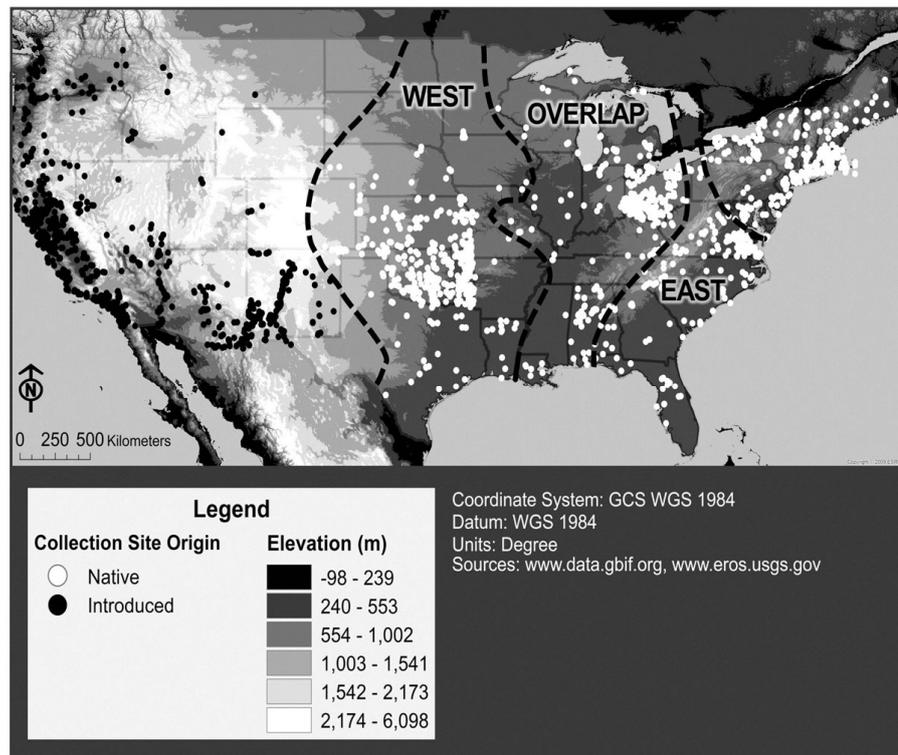
Skin darkening is a generalized response to UV-B across multiple taxonomic groups (Hairston 1976; Hansson 2000; Reguera et al. 2014), but it is unclear what the energetic costs of color change are. In amphibians, species-specific embryo pigmentation is often correlated with egg exposure to solar radiation (Blaustein and Belden 2003; Duellman and Trueb 1986). Larval skin darkening has also been quantified in several amphibian species in response to ambient and artificially enhanced UV-B exposure rates (Blaustein et al. 2001; Garcia et al. 2004). Body coloration is a highly plastic trait in most amphibian larvae, but color plasticity can often be constrained over ontogeny, with later-stage amphibian larvae adopting more consistent coloration patterns possibly due to differences in physiology (Sköld et al. 2012) or size-dependent selection pressures (Fernandez and Collins 1988; Garcia et al. 2003). Avoidance behaviors, such as preference for UV-B protected microhabitats, are also effective means for reducing UV-B damage (Belden et al. 2000; Garcia et al. 2004;

Marco et al. 2001). Further, when employed jointly in a correlated defense strategy, individuals may be capable of surviving extremely stressful UV-B environments (Garcia et al. 2009).

American bullfrogs (*Lithobates catesbeianus*) have successfully invaded approximately 40 countries across four continents (Lever 2003) and have become a serious conservation threat to native communities (Garner et al. 2006; Lowe et al. 2000). While no studies have quantified bullfrog response to UV-B, other ranid species suffer significant lethal and sub-lethal impacts from UV-B exposure (Blaustein et al. 1998; Tietge et al. 2001). Bullfrogs appear to have variable establishment success in UV-B intense environments, which may be due to differences in physiological and behavioral defenses. Introduced in Oregon for agricultural purposes in the early 20<sup>th</sup> century, bullfrogs have successfully invaded low elevation habitats throughout much of the Pacific Northwest. They were transported in 1938 to ponds in the Oregon Cascade Mountain Range (Cascades) as an ornamental species (J. Bowerman pers. comm.), but populations are rare above 1000m (Figure 1; Nussbaum et al. 1983). In contrast, stable breeding populations have been reported at high elevation sites in Venezuela, Argentina, China, and California (Moyle 1973; Hanselmann et al. 2004; Sanabria et al. 2005; Xuan et al. 2009). Understanding potential UV-B constraints on the bullfrog invasion in Oregon could provide important management information for the Western invasion range.

Our study explored invasive bullfrog tolerance and trait response to ecologically-relevant UV-B exposure rates found at low and high elevation amphibian breeding sites in Oregon. Bullfrogs are capable of plastic trait response to novel environmental stressors (Provenzano and Boone 2009; Relyea 2001), thus we hypothesized that bullfrogs would react to UV-B stress with darkening body coloration and increased refuge use. Further, this UV-B sensitivity may be size and stage dependent, with effects and trait responses varying over larval development. We tested three bullfrog populations from the Willamette Valley, OR. High elevation populations were not tested due to their rarity and our inability to locate more than one breeding site above 1000m. Our first experiment quantified larval survivorship and color change across two larval size classes (a proxy for ontogeny) using two UV-B exposure rates. These results informed the second experiment, where we tested for correlated trait response by quantifying color change and refuge use in

**Figure 1.** Map of documented bullfrog collection sites in the United States based on database records. Specimen localities obtained from coordinates of museum records from the Global Biodiversity Information Facility data portal. The native range was divided into four genetic groups based on nested clade analysis (Austin et al. 2004; Funk et al. 2010).



small-sized larvae at high and low UV-B conditions. We predicted that UV-B exposed bullfrog tadpoles would experience increased mortality when no refuge was available, with the magnitude of response dependent upon UV-B exposure level. We also predicted size-dependent color plasticity, with small-sized tadpoles exhibiting more intense body darkening relative to larger tadpoles. The smaller size class was also expected to combine increased refuge use with color change in high UV-B exposure treatments to maximize UV-B avoidance.

## Methods

We ran two experiments that quantified survivorship and color plasticity in American bullfrog tadpoles in response to UV-B radiation exposure as a function of size (experiment 1) and refuge availability (experiment 2). We tested three distinct low elevation populations in the Willamette Valley, OR; in pilot surveys we were only able to document breeding populations in one high elevation site in the Oregon Cascades and therefore restricted our study to low elevation populations (Garcia, unpubl. data). Newly hatched

(stage 25; Gosner 1960) and overwintered (stages 31 to 35; Gosner 1960) bullfrog larvae were collected from three lentic breeding sites in the Willamette Valley, OR (Adore pond, 44°40' 35.66"N and 123°12'45.07"W, 76.2m elevation; Lippy pond, 44°49'16.42"N and 123°11'55.57"W, 61m elevation; Dunn pond, 44°27'06.20"N and 123°22'56.72"W, 129m elevation). We selected these sites (separated by a minimum distance of 16.8 km) based on low likelihood of gene flow between populations. Sites had similar vegetation structure and depth profiles. Bullfrogs were collected 1–2 days prior to the start of each experiment. All individuals were transported to Oregon State University and held in an environmental chamber with constant temperature (26°C) and controlled photoperiod (12L:12D).

We used UV-B exposures corresponding to levels naturally found in the Willamette Valley, OR (~200m elevation; 8–10 $\mu$ W/cm<sup>2</sup>) and in the alpine region of the Oregon Cascades (~1500m; 20–24 $\mu$ W/cm<sup>2</sup>) where invasive bullfrog populations have been historically documented (Nussbaum 1983; Garcia et al. 2009). Filters were used to manipulate experimental UV-B exposure rates: Mylar filters excluded 95% of UV-B radiation

(control treatment) and high density polyethylene filters removed 15% of UV-B radiation. We achieved ecologically relevant sub-lethal high and low UV-B exposure treatments (Blaustein et al. 1998; Thurman et al. 2014) by applying high density polyethylene filters singly (high UV-B treatment) or doubly (low UV-B treatment). UV-B levels were measured with a UV-B probe (Solar Light Co., model PMA2100, Philadelphia, Pennsylvania, USA).

Color analysis was generated from digital photos of larvae staged in petri dishes (filled with approximately 2cm fresh water) on a white background. Standardized photos were taken at a fixed distance from the subject and light source with a Nikon D80 digital single-lens reflex camera. Images were analyzed using ImageJ 1.46 software (U.S. National Institutes of Health, Bethesda, Maryland, USA, <http://imagej.nih.gov/ij/>, 1997–2012). Amphibian larvae vary primarily in brightness values (amount of black versus white), with relatively constant chroma and hue values (Grill and Rush 2000; Garcia et al. 2009), therefore all images were converted to grayscale. We quantified brightness with black versus white pixel weights within a selected dorsal region on each larva: the body minus the tail region and behind the eye line. Brightness values were less impacted by internal structures in these regions. Larger brightness values indicate a lighter, paler color relative to smaller brightness values.

#### *Experiment 1 – Survivorship and color change across two size classes*

We categorized larvae into two size classes (proxy for age); the small size class consisted of newly hatched bullfrog larvae (average snout-tail body length =  $3.68 \pm 0.08$ cm) and the large size class was comprised of larvae that likely overwintered one year (average snout-tail body length =  $9.22 \pm 0.2$ cm). Phenologies and habitats were consistent across all three collection sites thus larvae were similar in size. We quantified survivorship and body color change using a  $2 \times 3 \times 3$  factorial design with two tadpole size classes (small, large), three populations (Adore, Lippy, Dunn), and three UV-B exposures (absent, low, and high) as treatment factors. Our lighting regime simulated natural conditions (Bancroft et al. 2008), with individuals exposed to full spectrum bulbs emitting negligible levels of UV-B during the first three hours and the last five hours of the light cycle (Vita-Life; Durotest Corporation, Fairfield, New Jersey, USA). During

peak UV hours (1100–1500hr), individuals were exposed to UV-B emitting bulbs (UV-313; Q-Panel Inc., Cleveland, Ohio, USA). Animals received a mean UV-B exposure rate of  $22.7 \pm 0.146 \mu\text{W}/\text{cm}^2$  in the high UV-B treatment,  $9.00 \pm 0.372 \mu\text{W}/\text{cm}^2$  in the low UV-B treatment and  $0.17 \pm 0.01 \mu\text{W}/\text{cm}^2$  in the UV-B control treatment.

The experiment had five replicates per fully-randomized treatment combination. Each of 90 experimental units (19cm  $\times$  32cm clear plastic tub) was placed on a white background and contained one bullfrog larva. All units were filled with 1500ml of de-chlorinated water, and water temperatures remained consistent regardless of UV-B exposure rates. The experiment ran for 24 full days in a UV-B chamber with constant temperature and photoperiod (26°C, 12L:12D). During each day of the experiment, survivorship was recorded and water levels were maintained. Larvae were fed algal pellets after each full water change, which was performed every third day of the experiment. To quantify plastic color response to UV-B treatments, digital photos of each individual were taken on the first day of the experiment at two distinct time points: prior to UV-B treatment exposure and after the 4 hour UV-B treatment exposure period.

Differences in survivorship among UV-B radiation exposure treatments, size classes, and populations were compared using Kaplan-Meier survival analysis (Kaplan and Meier 1958) in the ‘Survival’ package in R 2.15.2 (R Core Team 2012). When significant survivorship differences based on the Kaplan-Meier estimates were observed between groups, we conducted *post hoc* pairwise comparisons to further understand treatment effects. Following diagnostic tests of the assumptions of normality and equal variance, ANOVA was used to analyze change in body color after 4 hours of exposure to UV-B treatments (prior to any deaths). Pairwise differences in treatment means were tested using a Tukey HSD test in SYSTAT 11.1. In all cases, we used one-tailed criteria for tests where we had clear *a priori* hypotheses and an alpha of 0.05 as the indicator of statistical significance.

#### *Experiment 2 – Color change and refuge use*

We tested for correlated color change and refuge use responses using a  $3 \times 3$  factorial experimental design with three UV-B radiation exposures (absent, low, and high) across three populations of bullfrog larvae (Adore, Lippy, Dunn) using

**Table 1.** Chi square analysis using Kaplan-Meier estimates on survivorship over 24 days of UV-B exposure. Significant differences were detected between observed and expected survivorship probabilities across all three UV-B exposure groups ( $X^2=36.7$ ,  $df=2$ ,  $p=0.000$ ), and both size classes ( $X^2=10.8$ ,  $df=1$ ,  $p=0.001$ ).

UV-B Effect	N	Observed	Expected	(obs.-exp.) <sup>2</sup> /exp.	(obs.-exp.) <sup>2</sup> /N
Absent	24	6	18.90	8.81	17.06
Low	24	14	15.49	0.14	0.24
High	24	23	8.61	24.04	33.32
Size Class Effect	N	Observed	Expected	(obs.-exp.) <sup>2</sup> /exp.	(obs.-exp.) <sup>2</sup> /N
Small	36	27	16.9	6.05	10.8
Large	36	16	26.1	3.91	10.8

**Table 2.** Results of an ANOVA on effects of larval size, UV-B radiation and population on American bullfrog (*Lithobates catesbeianus*) body coloration. Color Difference is the difference in body coloration (brightness) before and after four hours of treatment exposure. Df = degrees of freedom, Asterisks indicate significance.

Response Variable	Effect	SS	DF	F	P
Color Difference	Size Class	6444.49	1	55.728	0.000*
	UV-B	491.73	2	2.126	0.129
	Pop	329.55	2	1.425	0.249
	Size × UV-B	711.45	2	3.076	0.054
	Size × Pop	58.87	2	0.255	0.776
	UV-B × Pop	265.04	4	0.573	0.683
	Size × UV-B × Pop	520.516	4	1.125	0.354
	Error	6244.64	54		

tadpoles from the small size class (average snout-tail body length =  $3.88 \pm 0.06$ cm). This experiment only tested the small size class as this was the treatment group that responded to UV-B radiation with color change (Results-Experiment 1). During the 4-hr peak UV-B exposure period, individuals received a mean exposure rate of  $23.3 \pm 0.339 \mu\text{W}/\text{cm}^2$  in the high UV-B treatment,  $9.29 \pm 0.309 \mu\text{W}/\text{cm}^2$  in the low UV-B treatment and  $0.15 \pm 0.01 \mu\text{W}/\text{cm}^2$  in the UV-B control treatment. Fully-randomized treatment combinations were replicated eight times for a total of 72 experimental units. Each unit (19cm × 32cm clear plastic tubs) contained one refuge consisting of an 8cm × 6cm piece of corrugated black plastic (bullfrog preference for this refuge type was confirmed in previous studies; Garcia et al. 2012). Placement of the refuge rotated clockwise around the four corners of the unit across all 72 units. All units contained 1500ml of filtered and dechlorinated tap water. Tadpoles were haphazardly selected from each population group and placed in a petri dish for pre-UV-B exposure digital photos. All individuals were then added to units and UV-B lights were turned on.

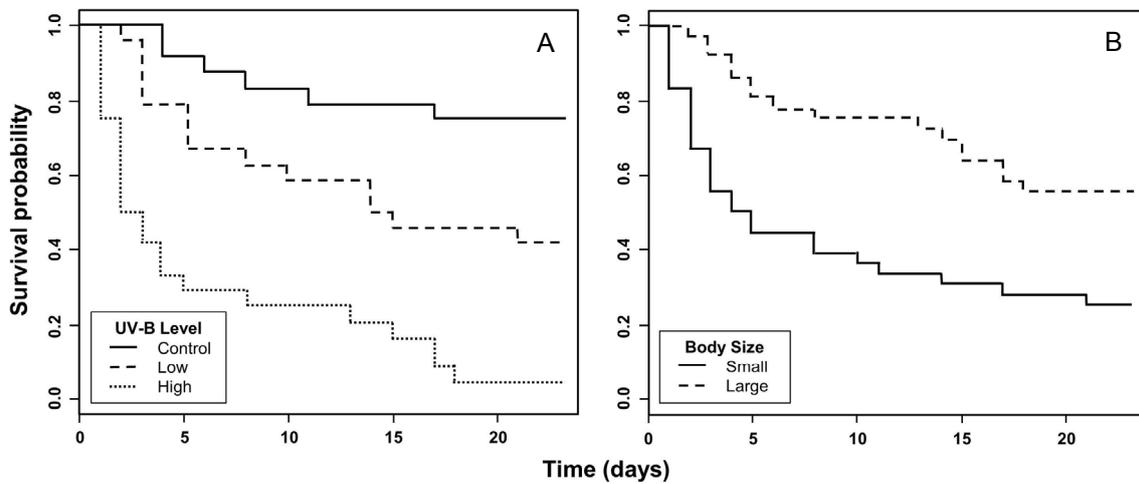
Bullfrogs were given a 20 minute acclimation period before behavioral observations began. Behavioral spot-checks and recording of refuge use were conducted every 20 minutes for a total

of 11 observations per unit beginning at 1100hr. The proportion of time spent in refuge across all 11 observations was calculated for all individuals. Observers were blind to assigned treatment combinations, and experimental units were located behind observation blinds to limit observer disturbance. After four hours of exposure to UV-B treatments, individuals were photographed again. We analyzed change in body color and proportion of time spent in refuge using one-way ANOVA with pairwise comparisons (Tukey HSD test) in SYSTAT 11.1.

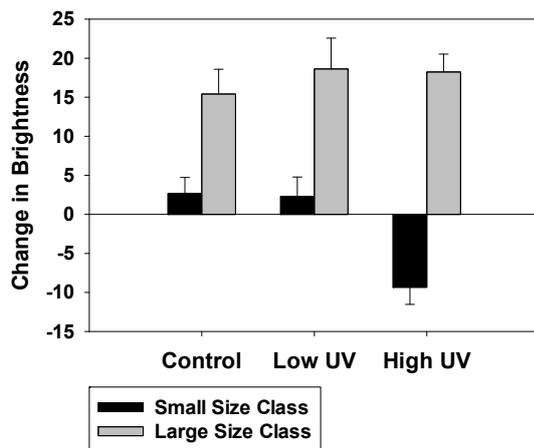
## Results

### *Experiment 1 – Survivorship and color change across two size classes*

We found that bullfrog larvae survivorship in both size classes was negatively affected by exposure to UV-B radiation. Both the low and high UV-B exposure treatments resulted in a significant reduction in survivorship over 24 days (Table 1; Figure 2-A). Pairwise comparisons indicated that survivorship differed significantly between the control and low UV-B groups ( $X^2=8.95$ ,  $df=1$ ,  $p=0.003$ ), control and high UV-B groups ( $X^2=32.84$ ,  $df=1$ ,  $p=0.000$ ), and low and high UV-B groups ( $X^2=24.18$ ,  $df=1$ ,  $p=0.000$ ). Further, size classes differed in probability of survivorship over time (Figure 2-B), with small



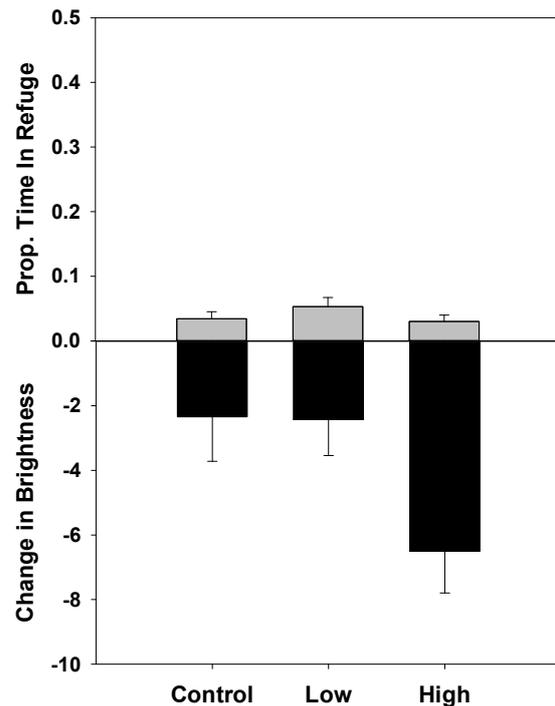
**Figure 2.** Kaplan-Meier survivorship functions over a 24 day experiment comparing survival probabilities among A) combined large and small larval size classes of *Lithobates catesbeianus* exposed to three UV-B radiation treatments: control, low, and high and B) large and small larval size classes. Significant differences were detected in survivorship probabilities among all three UV-B exposure groups ( $X^2=36.7$ ,  $df=2$ ,  $p=0.000$ ) and both size classes ( $X^2=10.8$ ,  $df=1$ ,  $p=0.001$ ).



**Figure 3.** Color change data for small and large size classes of larval *Lithobates catesbeianus* in response to UV-B radiation. Larger brightness values indicate a lighter color relative to smaller brightness values. Data are means  $\pm$  SE from color measurements taken after four hours of exposure to UV-B treatments.

larvae having a significantly greater mortality risk from UV-B radiation relative to large larvae ( $X^2=10.8$ ,  $df=1$ ,  $p=0.001$ ). Populations did not differ in their response to UV-B radiation within either larval size class (all  $p > 0.05$ ).

Only bullfrog larvae in the small size class exhibited a darkening of body color in response to high UV-B radiation exposure (Table 2; Figure 3). Low UV-B levels did not induce



**Figure 4.** Proportion of time spent in refuge (top panel) and change in larval *Lithobates catesbeianus* body coloration, or brightness, (bottom panel) after four hours of exposure to UV-B treatments.

a significant change in body coloration in larvae from the small size class, and no amount of UV-B radiation caused larvae from the large size class to darken. Conversely, these larger larvae became lighter in body color over time across all UV-B exposure treatments (Figure 3) likely due to the light colored background of the experimental unit (Garcia et al. 2003).

#### *Experiment 2 – Color change and refuge use*

We found that while larvae did become darker (Figure 4) over the 4 hour UV-B exposure period in the high UV-B treatment (UV-B effect:  $F_{2,68}=3.33$ ,  $p=0.042$ ), they did not correlate behavioral refuge use with changes in body coloration (UV-B effect:  $F_{2,68}=1.02$ ,  $p=0.366$ ). Larvae occupied refuge in less than 5% of the observation points (Figure 4), regardless of body coloration or UV-B exposure rate.

#### **Discussion**

We found that bullfrog larval survivorship was negatively impacted by UV-B exposure. Small and large-sized bullfrog larvae exhibited decreased survivorship when exposed to UV-B treatments mimicking naturally relevant low and high elevation exposure rates. Bullfrog larval mortality was correlated with the strength of the stressor, with individuals exposed to the high UV-B treatment having a lower survival probability relative to the low UV-B treatment. Further, the small size class had a lower survival probability when compared to the large size class. These results informed the second experiment, which tested only the small bullfrog size class for correlated UV-B defense strategies via darkening body coloration and increased refuge use. We did not find evidence supporting a correlated response. Larvae darkened in color after exposure to high UV-B conditions, but they did not spend time in refuge under any treatment condition.

These results reflect a size-dependent response to UV-B induced color change. This difference in color response indicates a size or age-related switch in functional trait response. Ontogenetic shifts in color response may be a function of seasonal variation in body coloration selection pressures (Van Buskirk 2011) or ontogenetic changes in color regulating hormone levels (i.e.,  $\alpha$ -MSH; Sköld et al. 2012). Previous studies have shown similar plasticity constraints over larval amphibian development (Fernandez

and Collins 1988; Alho et al. 2010). For example, late-stage *Ambystoma barbouri* and *A. texanum* salamander larvae exhibited decreased color plasticity in response to cold temperature conditions relative to early-stage, smaller-sized individuals (Garcia et al. 2003). Further, bullfrogs may alter their UV-B defense strategy over ontogeny as a function of size-dependent refuge availability. UV-B wavelengths attenuate with water depth (Kirk 1986), thus larvae can behaviorally escape UV-B by selecting deeper microhabitats. Risk from gape-limited predators in these deeper microhabitats, such as fish, is reduced as bullfrog larvae increase in body size (Garcia et al. 2012). Larval size may therefore be an indicator of a tradeoff between microhabitat use and color plasticity in this species.

We expected bullfrog larvae to scale UV-B response with risk intensity. While small-sized larvae darkened body color in the high UV-B exposure treatment, they still suffered increased mortality as a function of UV-B exposure. Therefore, a correlated defense strategy combining darkening with an additional defense, such as refuge use, could potentially protect larvae from UV-B-induced mortality. We found, however, that small-sized larvae did not respond with a correlated defense strategy when given access to refuge, even in highly stressful UV-B environments. Since bullfrog preference for the refuge material has been confirmed in a previous study by the authors (Garcia et al. 2012), this reluctance to utilize refuge may be explained by the presence of conflicting selection pressures (Bancroft et al 2008). Refuge use is costly as it prevents amphibian larvae from actively foraging, thus reducing growth and developmental rates and delaying time to metamorphosis (Werner and Anholt 1993). Small-sized bullfrog larvae may experience strong pressure to develop and grow quickly in order to reduce risk from predation by gape-limited predators or desiccation through habitat drying. These conflicting selection pressures could be limiting the ability of bullfrog larvae to effectively mediate UV-B damage. Further research is needed on susceptibility and trait responses to UV-B over ontogeny, especially since survival rates within different life stages (e.g., larvae vs. post-metamorph) can have distinct consequences on population dynamics (Garcia et al. 2006).

Alternative UV-B defenses may also account for the lack of color or behavioral responses in these invasive bullfrog populations. Enzymatic DNA repair or other physiological mechanisms can protect individuals from UV-B induced damage

(Blaustein et al. 1994) and may provide bullfrog larvae with a UV-B coping strategy while maintaining adequate growth and developmental trajectories. Photolyase activity, a highly efficient and error free photoreactivation enzyme, varies significantly across amphibian species and populations (Blaustein et al. 1994; Freidberg et al. 2006), with strong positive correlations between photolyase activity and UV-B resistance (Blaustein et al. 1994). Our results suggest that invasive bullfrogs in Oregon are utilizing alternative UV-B defense mechanisms such as increased depth choice or high photolyase activity to protect against ambient UV-B exposure due to ineffective color defenses.

Comparisons of bullfrog responses and sensitivity to UV-B across a broader invasion range could provide greater insight into the mechanisms allowing for the successful colonization of bullfrogs at globally disparate elevation ranges. The scarcity of breeding bullfrog populations in the Oregon Cascades is unique given the high elevational reach of bullfrogs in other invasion ranges (California, USA, Moyle 1973; Venezuela, Hanselmann et al. 2004; Argentina, Sanabria et al. 2005; China, Xuan et al. 2009). We posit that these international invasion ranges may have been colonized by bullfrogs from different source populations; these source populations may have experienced drastically different UV-B environments and exposure rates (Sol 2007; Cook et al. 2013). Bullfrogs in the Willamette Valley, OR have similar genetic profiles (cytochrome *b* haplotypes) to native bullfrog populations in the Great Lakes region and upper Mississippi River basin (the ‘overlap’ zone, Figure 1; Austin et al. 2004; Funk et al. 2010). The maximum elevation for populations occurring in the overlap zone is 944m (Global Biodiversity Information Facility 2012), and the mean elevation for these populations ( $233\text{m}\pm 111\text{m}$ ; Global Biodiversity Information Facility 2012) is similar to the Willamette Valley ( $196\text{m}\pm 178\text{m}$  average elevation; Branscomb 2002). Invasive Willamette Valley bullfrog populations are the likely stocking source for populations documented in the Oregon Cascades, and high elevation breeding sites experience UV-B exposure rates 2–3 $\times$  the intensities found at these potential source populations and low elevation OR sites ( $20\text{--}24\mu\text{W}/\text{cm}^2$  and  $8\text{--}10\mu\text{W}/\text{cm}^2$  respectively; Garcia et al. 2009).

In Europe, invasive bullfrog populations are also typically found in low elevation habitats (Ficetola et al. 2010) and the two dominant haplotypes match those from the ‘West’ and

‘East’ regions of the native bullfrog distribution ( $563\text{m}\pm 567\text{m}$  and  $233\text{m}\pm 249\text{m}$  mean elevations respectively; Ficetola et al. 2008; Global Biodiversity Information Facility 2012). Alternatively, populations in China can be found at 2700m and the dominant haplotype cannot be traced to a native bullfrog source, suggesting divergence or extirpation of the native lineage (Xuan et al. 2009; Bai et al. 2012). Stocking sources for bullfrog populations in South America are largely unknown, but bullfrogs in Venezuela can be found at relatively extreme elevations (2400m; Hanselmann et al. 2004). Likewise, populations are confirmed in six provinces of Argentina (Akmentins and Cardozo 2010), including San Juan (1800 to 2400m; Sanabria et al. 2005). Thus, bullfrog elevation restrictions in the Pacific Northwest invasion range could be a function of source populations with limited evolutionary exposure to high UV-B environments, and comparative research is needed to test this hypothesis.

Propagule pressure may also account for population differences in UV-B susceptibility as the number of initial colonizers and introduction events will influence persistence and evolutionary potential in novel environments (Gilchrist and Lee 2007; Lockwood et al. 2009). South America has the most extensive bullfrog invasion range relative to other continents, with expanding distributions associated with intensive frog farming in the late 20<sup>th</sup> century (Akmentins and Cardozo 2010; Hanselmann et al. 2004; Nori et al. 2011). China populations were also introduced for agricultural purposes circa 1950 (Liu et al. 2009) and many are actively maintained, thus propagule pressure is likely to be high (Liu and Li 2009). Europe has had several small-scale bullfrog introductions (<6 females) circa 1930 (Ficetola et al. 2008), and reduced propagule pressure may explain why these populations have not expanded into potentially suitable areas (Ficetola et al. 2010). Similarly, anecdotal evidence suggests that bullfrog introductions into high elevation Oregon Cascades sites occurred circa 1938 with small numbers of initial colonizers and extremely limited distributions (J. Bowerman, pers comm; Garcia, unpublished data).

Understanding bullfrog invasion potential in the Pacific Northwest invasion range is likely a combination of multiple factors (Bomford et al. 2008; Rago et al. 2012). We expect that bullfrog populations within and across global invasion ranges differ significantly in abiotic tolerances, introduction pathways, and propagule pressure. Our study is the first experimental test to quantify

bullfrog tolerance and trait response to UV-B stress. These data support the hypothesis that bullfrog populations in the Pacific Northwest invasion range are susceptible to UV-B damage, and this may be mitigating invasion potential in the Oregon Cascades. Future surveys are needed to document current distribution of high elevation bullfrog breeding sites throughout the Oregon and Washington Cascades and identify potential collection sites for direct population comparisons. Characterization of genetic divergence, stocking history, and abiotic tolerance for populations across elevation ranges would contribute significantly to the preemptive management of this biological invader.

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