Factors of global warming (UV-B radiation and temperature) affecting larval development of *Ambystoma granulosum*

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Abstract

Many stressors such as pollution, habitat destruction and currently global warming with the increase in UV radiationare are acting simultaneously on amphibians; this is also the case with climatic change for *Ambystoma granulosum.* In the present project the influence of increased temperature and UV radiation on the survival of larvae of A. granulosum was observed. Survival studies were conducted. In the third week of life of the amphibians, a significant difference (p-0.05) between the three temperatures 14, 19 and 25° C, and the five intensities of UV-B (0, 25, 50, 75 and 100%) were observed which was reflected in the somatic size. Individual/test in 14 °C show a longer time of hatching with respect to individual/test under the other two temperatures. An increase of 50% in hatching time between the temperatures of 14°C to 25°C in a maximum radiation (100%) was observed. Considering that central México registers the highest radiation in the area, *A. granulosum* is vulnerable to increases in the temperature and exposure to radiation, which is true not only for this species but also for other species of the genus Ambystoma.

Key words: *Ambystoma granulosum*, global warming, UV-B radiation, temperature increase, axolotl.

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Factores del calentamiento global (radiación UV-B y temperatura) que afectan el desarrollo larvario de *Ambystoma granulosum*

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Resumen

Muchos factores de estrés, como la contaminación, destrucción del hábitat y actualmente el calentamiento global con los incrementos en radiaciones UV actúan simultáneamente sobre los anfibios, este también es el caso de los cambios climáticos para *Ambystoma granulosum.* En el presente trabajo se observó la influencia de temperaturas más altas y radiación UV en la sobrevivencia de larvas de *A. granulosum.* Se llevaron a cabo estudios de sobrevivencia. En la tercera semana de vida de los anfibios se observó una diferencia significativa (p-0.05) entre las temperaturas de 14, 19 y 25 °C y las cinco intensidades de UV-B (0, 25, 50, 75 y 100%) que se reflejaron en la dimensión somática. La prueba individual a 14 °C mostró un tiempo más largo para eclosionar en comparación con las pruebas individuales en las otras temperaturas. Se observó un incremento de 50% en el tiempo de eclosionado entre las temperaturas de 14 a 25 °C bajo una radiación máxima (100%). Considerando que la región central de México registra la mayor radiación del área, *A. granulosum* es vulnerable a los incrementos de temperatura y exposición a radiación, un hecho que tiene validez tanto para esta especie como para otras del género Ambystoma.

Palabras clave: *Ambystoma granulosum*, calentamiento global, radiación UV-B, incremento de temperatura, axolotl.

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Introduction

The loss and extinction of species is occurring rapidly (Kaufman, 1992: 12; Frías-Alvarez et al. 2010a: 19; Hansen et al. 2012: 22; Breed et al. 2013: 3). The causes for the declination of the amphibian population have been determined to be the loss of habitat (Harper et al. 2015: 13). Along with habitat destruction, the most significant factors contributing to amphibian population declines and extinction are pollution, introduced exotic species, disease, climate change and atmospheric processes associated to global warming such as increase of temperature and UV-radiation. All these causes may have interacting co-factors (Blaustein et al. 2011: 16). Many stressors are acting simultaneously on amphibians, and in specific regions, one factor may be more devastating than others (Hussain, 2012: 9); as in the case of climate change and increased ultraviolet radiation (Parmesan & Matthews, 2006: 41).

The interest around amphibians is partly due to their value as indicators of disturbances to ecosystems (Hölting et al. 2016: 1). Amphibians have moist, permeable skin that is directly exposed to water and sunlight (Blaustein et al. 2012: 16). Furthermore, amphibians may be considered preys, predators or herbivores in many ecosystems (Ripple et al. 2014: 1). The loss of amphibians affects the trophic dynamics, and consequences can have a cascading effect on other organisms (Costa & Vonesh 2013: 7).

It has been observed that UV-B radiation, which comprises of 290-320 nm can be highly negative for species of amphibians, particularly in open areas where radiation is more direct (Häder, 2012: 1). It can cause cell death, as well as mutations and deformities, slow growth rate of individuals, damage in the immune system, and induction of cumulative damage that can be lethal (Garcia et al. 2006: 6; Croteau et al. 2008: 18; Blaustein et al. 2010: 16; Agostini et al. 2013: 8; Tapley et al. 2015: 6).

Additionally, this radiation generates a lot more heat in oviposited eggs, thus inducing an exclusion and early development (Alix, 2013: 152). Also, the increase of temperature accelerates the process, such damage has been observed in the wild and in laboratory by several researchers (Crump, 2001: 19; Croteau et al. 2008: 18). The elevated UV radiation and temperature causes low success in the hatching of larvae of amphibians, as can be found in some genus such as *Ambystoma* (Belden et al. 2000: 6); *Triturus cristatus* (Mačát et al. 2015: 6); *Litoria* and *Crinia* (Broomhall et al. 2000: 7); *Hyla* and *Taricha* (Ovaska et al. 1997: 7; Blaustein et al. 2000: 16); *Rana* (Marquis & Miaud, 2008: 8) and Lissotriton (Galloy & Denoël, 2010: 4).

Likewise, available data shows that exposure to UV-B in adults causes physiological damage (Thurman et al. 2014: 10), slow development (Kern et al. 2014: 6; Yu et al. 2014: 5) and abnormalities during all stages of development (Fischer and Phillips 2014: 8); as well as alterations in the behavior of some amphibians (Thurman et al. 2014: 10). Despite this, sometimes exposure during the embryonic or larval stages may not show effects; such is the case of *Rana temporaria* (Muir et al. 2014: 13).

Mexico has 375 identified species of amphibians and thus has the fifth richest amphibian fauna in the world (Botello et al. 2015: 6). Recent studies show that the populations of amphibians in Mexico are declining due to global warming (Caviedes-Solis et al. 2015: 11; Ochoa-Ochoa et al. 2012: 8; Quintero et al. 2014). *Ambystoma mexicanum* is an endemic species, and it is emblematic of the Mexican culture (Frías-Alvarez et al. 2010a: 19; Chaparro-Herrera et al. 2011: 8). This species appears on the red list of the International Union for Conservation of Nature (IUCN) as Critically Endangered (CR) (Roth and Obringer 2003;IUCN 2009: 15), and the natural populations of *A. mexicanum* have decreased by 60% (Frías-Álvarez et al. 2010b: 43).

Recent research has documented the causes of loss and declines in amphibian populations specifically related to *A. granulosum*, where the focus has been on reproductive biology, genetics, ecotoxicology and the current status of the species in their natural environment (Park et al. 2004: 12; Frías-Alvarez et al. 2010b: 43; Shoots et al. 2015: 8; Hinman 2016). Such studies show that the mechanisms that underlie the decrease in amphibian populations are complex, involving interactions between abiotic and biotic components of the ecosystem (Cahill et al. 2012). UV radiation has only been studied in embryos (Frias-Alvarez et al. 2010a: 19), however, no study has evaluated the impact of increased UV-B radiation on the survival and growth of somatic larvae during the first weeks after hatching.

This paper will briefly review the effects and implications of two factors that contribute to the development and survival of the genus *Ambystoma*, amphibians endemic to North America especially with a greater number of species in central Mexico (Chaparro-Herrera et al. 2013: 8): Increase in ultraviolet radiation (mainly UV-B radiation) and temperature change, both major factors of global warming (Williamson et al. 2014: 7). Our main focus is on UV radiation and its interactions with increased temperatures, since other studies have shown separate effects in different amphibians (Frías-Álvarez et al. 2010a: 19; Smith et al. 2015: 6).

Materials and Methods

Fertilized eggs (about 400 of a single female) from *Ambystoma granulosum*, were obtained from the Laboratory of Herpetology at the Facultad de Estudios Superiores Iztacala of the National Autonomous University of Mexico, they were incubated at 15 to 18°C to a light: dark regime of 12:12 hrs. These individuals have been maintained under laboratory conditions for the past two years. About 3% of the eggs failed to hatch during the first week and nearly 20% of the hatched larvae died during the second week. All those that survived beyond this period were able to survive until the end the study period. Larvae were maintained using moderately hard water as the medium (EPA medium), which was prepared by dissolving 0.095 g NaHCO3, 0.06 g CaSO4, 0.06 g MgSO4, and 0.002 g KCl in one liter of, distilled water (Anonymous, 1985). The larvae were placed in shallow transparent trays and were fed ad libitum on a mixture of rotifers and cladocerans.

Temperature and UV-B Radiation V Experiments

The experiments of the effects of temperature and UV-B in the granular salamander developmented larvae were carried out during a period of 57 days without interruption, until the organisms in treatments died. Daily results were obtained after the experiments showed survival analysis of organisms and once every week the growth and development of individuals were analyzed. At the beginning of the experiment, four *A. granulosum* eggs with embryos were added to containers of 250 ml of capacity, containing 50 ml of the EPA with microcrustacean food (cladocerans) ad libitum. The factors that were analyzed were temperature and UV-B, therefore, the eggs were placed at three temperatures 14°C, 19°C and 25°C without UV-B radiation, and randomly placed in the combination of temperatures with UV-B radiation. There were four replicates per treatment. Exposure to UV-B radiation treatment temperature was regulated by placing a protection mesh on each container with different mesh openings (0.9 mm, 1.2 mm and 1.5 mm) simulating the penetration of ultraviolet rays in a body of water with suspended particles. UV treatments were the following: five intensities of UV radiation (0, 25, 50, 75 and 100%) seeking to verify the turbidity or the presence of organic particles to reduce the effects of ultraviolet rays. Ultraviolet light was applied with a lamp emitting a wavelength of 280 nm. Ultraviolet light was applied in periods of 12:12 simulating sunlight period on larvae of *A. granulosum.*

In experiments, hatching time according to the treatment, survival time of organisms hatched, the size and development of the larvae, were the factors analyzed.

Effects in hatching time

Hatching times of 192 eggs of *A. granulosum* were analyzed and used a day after oviposition. The eggs were placed in the treatments described above. Daily monitoring and larval hatching times were recorded. Similarly, the record of collapsed eggs that failed to hatch was kept.

Effects on survival

For comparative studies the surviving individuals were counted daily. A group of larvae of the same oviposition were thus of the same age. To calculate the survival table (lx) the values of the number of daily survivors was used. In the same survival table, the value of mortality was immediately obtained since the number of larvae deaths per day is given from survivors in treatment observing the effect of temperature, UV-B radiation, and the mixture of these other individuals, expressed as the difference:

$$dx = lx - lx + 1$$

Effects on somatic growth

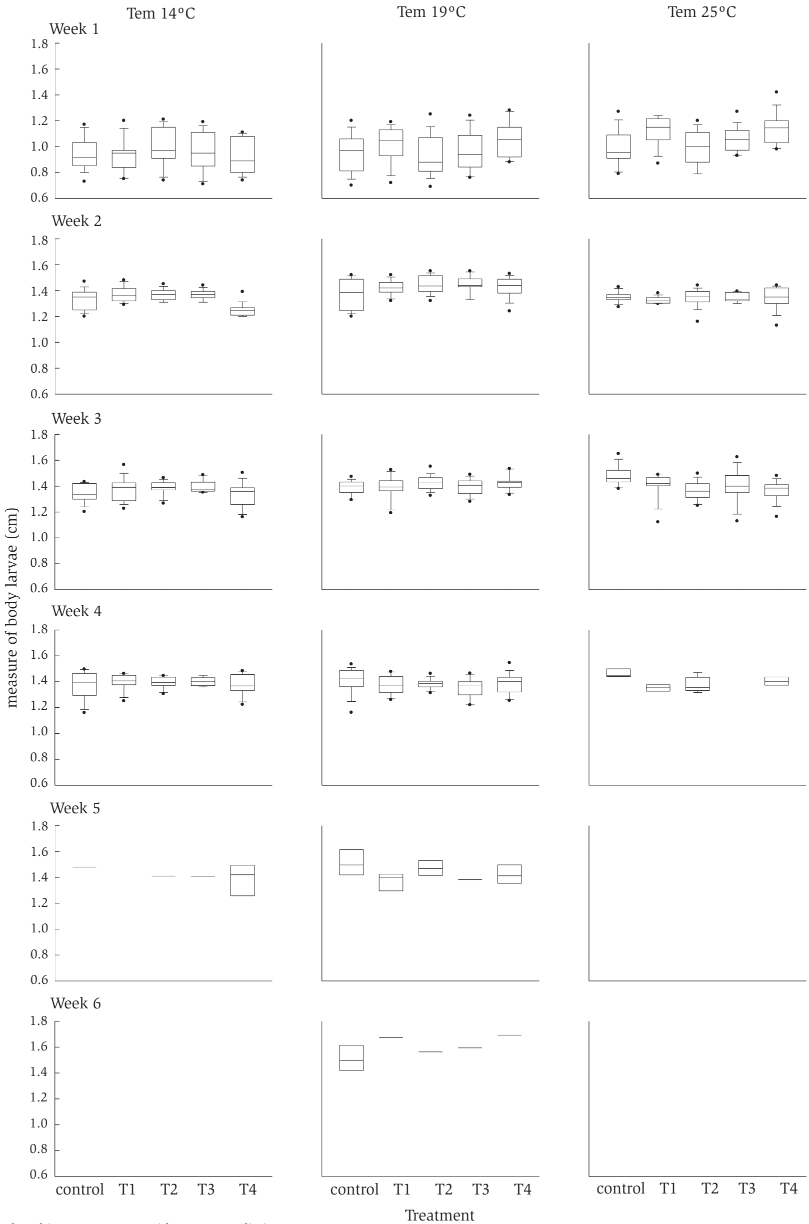
Every week in a 57 day period: A) the period of hatching was observed, and somatic embryos size measured. B) In each treatment, after hatching, larvae were measured each week while they were alive.

Data from hatching time, survival, and somatic growth experiments were statistically analyzed using analysis of variance (ANOVA) (Sokal & Rohlf, 2000). Post hoc (Holm Sidak) analysis was used for multiple comparisons utilizing the software Sigma plot ver. 11.

Results

Effects of UV-B radiation and temperature on development of Ambystoma granulosum larvae

The results of the effect of temperature and UV-B radiation on somatic growth are graphed in Figure 1. In the third week, there is a significant difference (two-way ANOVA, $P < 0.001$) (Table 1) between the three applied temperatures (14, 19 and 25°C) and the five intensities of UV-B (0, 25, 50, 75 and 100%). The significant differences in temperatures were between 14°C vs 19 and 25°C; and between 19 vs 25°C (Holm Sidak -0.05) (Table 1). The significant differences in sizes of the larvae of *A. granulosum* were observed between the treatment of 100% exposure to UV-B radiation treatments with 0, 25, 50 and 75% of UV-B exposure (Holm-Sidak 0.05) (Table 1). Average sizes in the third week at 14°C 19°C and 25°C were 1.37, 1.41 and 1.45 cm respectively. Treatment in 19°C and 50% of UV radiation in the third week registered the greatest size of 1.56 cm. It is important to emphasize that during the second and third weeks the differences were observed in all treatments, according to Holm Sidak test (Table 1).



Control-Ambient temperature without UV-B radiation
T1 - 25% uv T2 - 50% uv T3 - 75% uv T4 - 100% uv

FIGURE 1. The effect on the somatic size of three temperatures (14, 19 y 25 °C) and five intensities of UV radiation (0, 25, 50, 75 y 100%) in larvae of *A. granulosum*, from egg to hatching and development over a period of six week

Table 1. Two way ANOVA on the effect radiation UV-B and temperature in development by larvae *A. granulosum*, during the first four weeks

Source of variation	DF	SS	MS	F	P	Normality Test	Homes-Sidak (0.05) diff. significant
Week 1							
Temperature	2	0.105	0.0524	5.539	0.005	p = 0.696	1 vs 3
UV-B	4	0.141	0.0282	2.976	0.013		4 vs 8
Residual	160	1.514	0.0094				
Total	167	1.835	0.0110				
Week 2							
Temperature	2	0.427	0.213	122.903	<0.001	p = 0.053	1 vs 2; 2vs 3; 1 vs 3
UV-B	4	0.024	0.006	3.577	0.008		6 vs 8
Residual	169	0.294	0.0017				
Total	183	0.986	0.0053				
Week 3							
Temperature	2	0.174	0.087	28.602	<0.001	p = 0.093	No diff. significant
UV-B	4	0.025	0.006	2.114	0.082		4 vs 8; 5 vs 8; 6 vs 8; 7 vs 8
Residual	145	0.442	0.003				
Total	159	0.757	0.004				
Week 4							
Temperature	2	0.017	0.008	2.749	0.068	p = 0.634	No diff. significant
UV-B	4	0.010	0.002	0.797	0.530		
Residual	117	0.376	0.003				
Total	123	0.407	0.003				
Week 5							
Temperature	1	0.0001	0.001	0.049	0.826	p = 0.826	No diff. significant
UV-B	4	0.0303	0.0075	1.931	1.139		
Residual	23	0.0903	0.0039				
Total	28	0.0121	0.0043				

DF = degrees of freedom, SS = sum of squares, MS = mean square, F = F-ratio, * = only treatment 1 (14˚C) and treatment 2 (19 ˚C) decline treatment 3 (25 ˚C)

Effects of UV-B radiation and temperature in population growth on Ambystoma granulosum larvae

A significant difference in the survival of the larvae of *A. granulosum* was observed between the applied temperatures (14, 19 and 25°C) and the five UV radiation intensities (0, 25, 50, 75 and 100%) $P < 0.001$ (two-way ANOVA) (Figure 2; Table 2), mainly between temperatures 14 and 25°C (Holm-Sidak 0.05) (Table 2). Significant differences between UV treatments were between

0% exposure to 25, 50 and 100% (Holm-Sidak 0.05) (Table 2). The larvae of *A. granulosum* registered increased survival in 19°C with 0% UV-B of 48 days, on the other hand, a survival of 14 days at 25°C in 100% UV-B was registered. Meanwhile, in a temperature of 14°C with 100% radiation a survival of 21 days

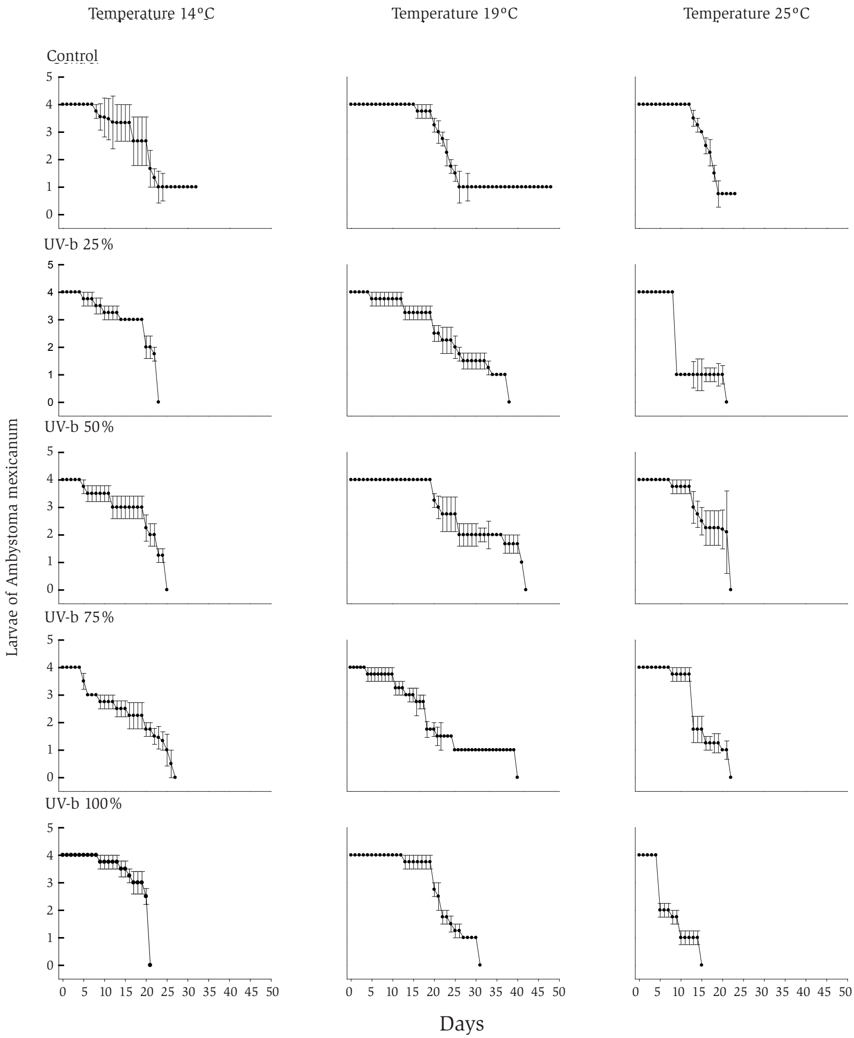


Figure 2. Effect on the survival of larvae of *A. granulosum* exposed to three temperatures (14, 19 y 25 °C) and five intensities of UV radiation (0, 25, 50, 75 y 100%) from egg to hatching and development over a period of six weeks

was registered (Figure 2). In the three temperatures (14,19 and 25 °C) exposure of 75% of UV-B registered 27, 47 and 21 days of survival respectively (Figure 2). In 19°C the organisms remained constant and increased survival was observed.

Table 2. Two way ANOVA on the effect radiation UV-B and temperature in the survival of larvae of *A. granulosum* in the first five weeks after hatching

Source of variation	DF	SS	MS	F	P	Normality < Test	Homes-Sidak (0.05) diff. significant
Temperature	2	5.389	2.695	1.997	<0.137	p 0.050	1 vs 3
UV-B	4	15.957	3.989	2.956	0.020		4 vs 5; 4 vs 8; 5 vs 6
Temperature	8	37.392	4.674	3.463	0.001		
x UV-B							
Residual	426	574.889	1.350				
Total	440	642.751	1.461				

DF = degrees of fredom, SS = sum of squares, MS = mean square, F = F-ratio, * = only treatment 1 (14˚C) and treatment 2 (19 ˚C) decline treatment 3 (25 ˚C); treatment uv-b: 4 (without uv-b), 5 (25%), 6 (50%), 7 (75%) and 8 (100%). Normality test P < 0.050

Effect of temperature and UV radiation on the larval hatching time of Ambystoma granulosum.

The various hatching times between treatments (see Figure 3), show that the shortest hatching time was registered at 25°C to 100% of UV-B radiation. A longer hatching time is apparent with respect to the other two temperatures (19 and 25°C). Hatch time increases by 50% between 14°C to 25°C in a maximum radiation (100%). On the other hand, there is a difference in hatching time between 14°C and 19°C of 15.79% with the 100% radiation exposure.

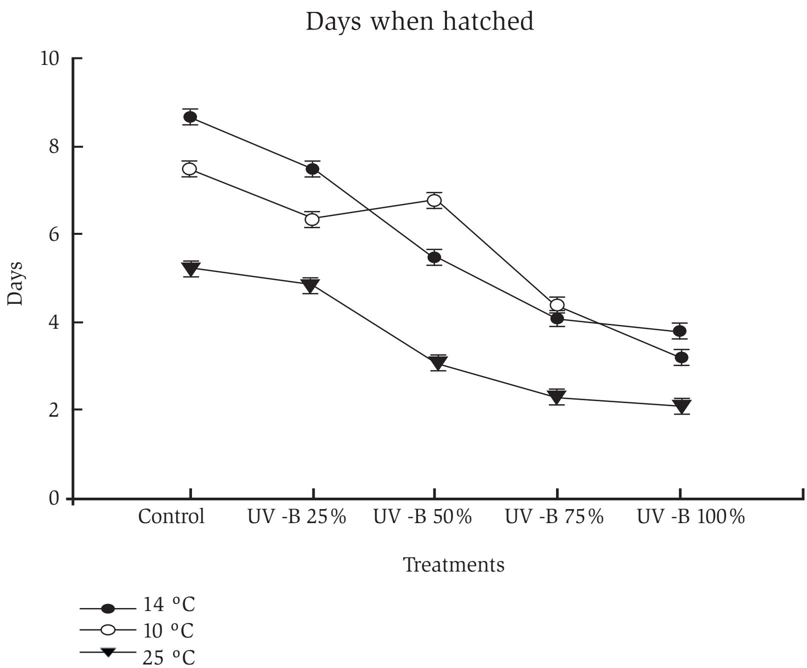


Figure 3. Hatching time of *A. granulosum* larvae exposed to three temperatures (14, 19 y 25 °C) and five intensities of UV radiation (0, 25, 50, 75 y 100%) from egg to hatching and development over a period of six weeks

Discussion

The present study found that *Ambystoma granulosum* larval survivorship was negatively impacted by UV-B exposure. Small and large-sized *A. granulosum* larvae exhibited decreased survivorship when exposed to UV-B treatments, mimicking naturally relevant low and high elevation exposure rates. Larval mortality was associated with the stressor, and individuals exposed to the high UV-B treatment displayed a lower survival probability relative to the low UV-B treatment. Furthermore, the smaller sizes had a lower survival probability when compared to the larger sizes (Pilliod et al. 2010: 8). Larvae exposed to 100% UV-B and to 14°C, displayed a slow somatic growth averaging 1.4 centimeters in the fifth week, compared with larvae at zero radiation, which reached an average of 1.5 centimeters, but they failed to survive beyond 21 days.

Ultraviolet-B radiation combined with temperature had a large negative effect on both survival and growth in the analysis. Some studies of the effects of UVB on aquatic organisms generally suggest that the effects of UV vary widely among organisms (Oromi et al. 2015: 6; Banaszak & Lesser 2009: 18; Tomanek, 2010: 8; Dahms & Lee 2010: 11; Häder et al. 2011: 18). The data from this work concludes that UVB radiation has a limited effect on survival. Less than half (45%) of the original comparisons observed a significant effect of UVB radiation on survival. The analysis of the effects of UVB radiation on growth would detect a negative effect, as mentioned in most of the studies that reported a significant effect on growth (Häder et al. 2011: 18). These analyses have broad trends that may be obscured by variation and poor statistical power. Significance level depends on both the size of the measured effect and the sample size of each treatment (UV-B and temperature) (Durlak, 2009). Thus, two factors measuring the same effect may have different statistical outcomes simply due to different sample sizes. These large effects on sizes may be due to extreme experimental conditions or indicate that these organisms are particularly susceptible to damage from UVB radiation. The present analysis suggests that the effect varies among organisms, but the effect tends to be large and negative. The negative effect of UVB on both survival and growth was detected despite different percentages of UV-B.

The climate with temperature and UV-B as variables can determine the distribution, growth, productivity, and reproduction of plants and animals (Häder et al. 2011: 18). Changes in hydrology can influence species in a variety of ways, but the most completely understood processes are those that link temperature with UV-B in the size of aquatic species (Palmer et al. 2009: 15), and consequently a low survival rate was verified in the results of this project. The changes in climate, mainly in temperature and UV-B radiation indicate that the coming decades will have diverse effects on several species especially the ones of the aquatic life, such as amphibians (Parmesan & Matthews, 2006:41; Palmer et al. 2009: 15; Tomanek, 2010: 8; Frías-Alvarez et al. 2010b: 43; Häder et al. 2011: 18; Häder, 2012:23; Hussain, 2012: 9; Ochoa-Ochoa et al. 2012: 8; Thurman et al. 2014: 10; Smith et al. 2015: 6).

In experiments with *A. granulosum* larvae, a significant difference was observed in survival and growth, suggesting that the magnitude of the effect of

UVB radiation can be predicted by percentage of UV-B radiation to which the organism have been exposed. Likewise, the combination of temperature may increase or decrease the effect on both larval size and mortality. For example, shorter body size measurements were recorded in treatments at a temperature of 25°C, compared to treatments of 14 and 19°C. Furthermore, adding to the effect that temperature has on larvae of *A. granulosum* in body size, a shorter survival period was observed in 25°C at 100% UV-B, reaching an average 15 days, compared to a survival of 30 days at 100% radiation UV-B but with a temperature of 19°C. Therefore, it can be stated that when aquatic organisms such as *A. granulosum* larvae that are exposed to direct radiation at 100% UV-B in a temperature of 19°C, would have a 50% mortality rate within 15 days if the temperature were to be increased by six degrees celsius.

The present work also analyzed the effect of temperature in the embryonic development of *A. granulosum*, and the results suggest that the metabolism of embryos is faster at higher temperatures as observed in Figures 1 and 2. This reasoning does not deal with the question of why they develop slower in cold temperatures. It could be said that the rapid development of the larvae of *A. granulosum* is not clear and that the fastest development at high temperatures is adaptive. The presence of vitellus in eggs for a longer time in cooler temperatures may be related to protection; the vitellus protects embryos from a variety of biotic and abiotic threats, including predation, infection, extreme salinity, heat, desiccation and ultraviolet radiation (Gualandris-Parisot et al. 2002: 8; Marquis et al. 2006: 3; Rollins-Smith, 2009: 6; Baier et al. 2016). Moreover, in colder temperatures eggs can better protect embryos at the same time without causing a lack of oxygen (Shu et al. 2015: 11).

The reproduction of several species of *Ambystoma* takes place mainly in the cold months in which the temperature ranges from 10 to 15°C, as is the case of *A. granulosum* (Recuero et al. 2010: 15). As described in the Figure 3, it can be stated that the organism reproduces in cold months as a survival strategy. It has been observed that in some amphibian species like *Rana sylvatica*, *R. palustris* and *R. pipiens* that the egg-mass is thicker when found in cold water (10-15°C) (Woods, 1999: 8), while the egg-mass of two species whose breeding takes place primarily in summer, *R. clamitans* and *R. catesbeiana* in water temperatures of about 25°C, are slimmer (Tweedell, 2013). This results in speedy hatching and larval development. Currently, increased temperatures on the planet is affecting several bodies of water (Gunderson et al. 2016: 21), and as indicated in the present project, the temperature increase will have a negative effect on the larvae of *A. granulosum* eggs, its hatching, and development in the first weeks of its life.

Arrighi et al. (2013) showed in a study developed by the principles of neural tube, that the rate of development of amphibian embryos of *Bombina orientalis* was much lower at temperatures below 16°C and 14°C. The results in this proyect show that the hatching of larvae of *A. granulosum* was slower at temperatures of 14 and 19°C (Figure 3). The amphibian embryos in the range of constant temperatures between 14 and 36°C show norms for the thermal reaction stage of development (Arrighi et al. 2013). Under these treatments a pattern was observed allowing the comparison of the hatching in the control groups at the temperatures 14, 19 and 25°C with 0% exposure to UV radiation, where hatching at 19°C took place on average during the sixth day, and on the seventh day at 14°C. At the highest temperature (25°C) the hatchlings were found on the fifth day on average. Mečaková and Gvozdik (2009:6) explored the effects of temperature in the hatching of amphibian (newt European) *Triturus alpestris*, and they found that at cooler temperatures the speed of the embryogenesis and hatching develops at a slower rate. Therefore, the previous results (and the results presented here) show the effects of embryonic development in the amphibians under a specific temperature in an environment where a range of temperatures is experienced, affecting its stages of development.

Studies performed in some species have found that the effects of temperature depend on the magnitude of this variation combined with other environmental factors such as UV radiation (Parmesan and Matthews, 2006:41; Palmer et al. 2009: 15; Tomanek 2010: 8; Häder et al. 2011: 18; Ochoa-Ochoa et al. 2012: 8; Breed et al. 2013: 3; Fischer & Phillips, 2014: 8; Williamson et al. 2014: 7; Gunderson et al. 2016: 21). But until now the relationship between temperature and UV radiation has not been tested or studied and converted to non-linear whithin the limits of species with a range of thermal tolerance as *A. granulosum*, and existing models predict that temperature fluctuations should be of ecological importance (Arrighi et al. 2013).

By combining the effects of UV radiation observed that demonstrate that on average hatchings at a temperature of 25°C and an exposure of 100% of UV radiation hatchings were observed on the second day.

Moreover, under a 100% UV radiation and with 19°C on average, hatching was first observed on the third day, and on the fifth day at 14°C under the same conditions of UV radiation exposure.

The amphibians have complex life cycles and respond to environmental conditions through altering the times of transition between stages of life, thus resulting in a wide range of sizes at different stages that have effects on adult phenotypes and their ability to endure (Tarvin et al. 2015:8). However, little is known about how growth is related to environmental conditions after hatching. In this study, it was observed that the smaller individuals of *A. granulosum* were registered in temperatures of 14 and 19°C (Figure 1), for example in one week on average larvae median ± 1cm at a temperature of 14°C, while at a temperature of 25°C on average 1.2 cm ± mediate.

Tarvin et al (2015: 8) comment that organisms that develop with larger sizes at the beginning of hatching have a greater opportunity to feed and as a consequence show increased activity related to growth, which suggests that the biggest metamorphs could compromise growth to improve survival. But in this syudy, higher mortality rates were observed in organisms that remained at 25°C, which were the larger size organisms. On average, in temperatures of 14 and 19°C the registered survival rate was between 30 and 47 days respectively, while at 25°C, organisms did not survive more than 20 days (Figure 2). It is not known if temperature has an effect on the structured thickness of the egg mass or if the cells assume different conformations. What it is known is that some egg masses avoided diffusion limitations through generating currents of driven convection by heat through interstitial channels (Mills & Ward, 2015: 4).

Ever increasing UV-B radiation and temperature as resulted by the current climate change (Wong et al. 2015) together with what was observed in this work shows that these factors limit the survival and development of *A. granulosum* since they accelerate the time required for hatching and growth. This has a significant effect on survival because it exposes the weak and underdeveloped larvae. Considering that the highest radiations is in central Mexico (Parra-Olea et al. 2005: 6), and that is the current area of distribution of *A. granulosum*, the species vulnerable to increases in temperature and exposure to radiation, which is probably true not only for this species but also for other species of the genus *Ambystoma*.

Overall, the consequences amphibians are experiencing, particularly in the genus Ambystoma (species endemic to Mexico) as is the case of *Ambystoma granulosum* with respect to the effects of climatic change and the increase of UV radiation in the central zone of Mexico, where this species is found, can be reported. The temperature increase directly influences the embryonic and larval development of *A. granulosum*, accelerating growth and consequently maturation in larvae. On the other hand, the increase of UV radiation influences the survival of larvae of these amphibians.

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References

Agostini, M. G., Kacoliris, F., Demetrio, P., Natale, G. S., Bonetto, C. and Ronco, A. E. (2013). Abnormalities in amphibian populations inhabiting agroecosystems in northeastern Buenos Aires Province, Argentina. Dis. *Aquat. Org.* 104: 163-171.

Alix, D. M. (2013). *Influence of land use change on amphibian species assemblages and larval development* (Doctoral dissertation, Auburn University).

Anonymous. (1985). *Methods of measuring the acute toxicity of effluents to freshwater and marine organisms*. US Environment Protection Agency EPA/600/4-85/0 13.

Arrighi, J. M., Lencer, E. S., Jukar, A., Park, D., Phillips, P. C. and Kaplan, R. H. (2013). Daily temperature fluctuations unpredictably influence developmental rate and morphology at a critical early larval stage in a frog. *BMC Ecol*. 13: 1.

Baier, F., Jedinger, M., Gruber, E. and Zaller, J. G. (2016). Temperature seems to be an important factor when assessing effects of a glyphosate-based herbicide on egg and tadpole growth of Common toads (*Bufo bufo*; Amphibia L.). *Front. Environ. Sci*. 4: 51.

Banaszak, A. T. and Lesser, M. P. (2009). Effects of solar ultraviolet radiation on coral reef organisms. *Photochem. Photobiol. Sci*. 8: 1276-1294.

Belden, L. K., Wildy, E. L. and Blaustein, A. R. (2000). Growth, survival and behaviour of larval long-toed salamanders (*Ambystoma macrodactylum*) exposed to ambient levels of UV-B radiation. *J. Zool.* 251: 473-479.

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*). *Ethol.* 106: 227-234.

Blaustein, A. R., Walls, S. C., Bancroft, B. A., Lawler, J. J., Searle, C. L. and Gervasi, S. S. (2010). Direct and indirect effects of climate change on amphibian populations. *Divers*. 2: 281-313.

Blaustein, A. R., Han, B. A., Relyea, R. A., Johnson, P. T., Buck, J. C., Gervasi, S. S. and Kats, L. B. (2011). The complexity of amphibian population declines: understanding the role of cofactors in driving amphibian losses. *Ann. N. Y. Acad. Sci.* 1223: 108-119.

Blaustein, A. R., Searle, C., Bancroft, B. A. and Lawler, J. (2012). Amphibian population declines and climate change. *Ecological Consequences of Climate Change: Mechanisms, Conservation, and Management,* 30-46.

Botello, F., Sarkar, S. and Sánchez-Cordero, V. (2015). Impact of habitat loss on distributions of terrestrial vertebrates in a high-biodiversity region in Mexico. *Biol. Cons.* 184: 59-65.

Breed, G. A., Stichter, S. and Crone, E. E. (2013). Climate-driven changes in northeastern US butterfly communities. *Nat. Clim. Change.* 3: 142-145.

Broomhall, S. D., Osborne, W. S. and Cunningham, R. B. (2000): Comparative Effects of Ambient Ultraviolet-B Radiation on Two Sympatric Species of Australian Frogs. *Cons. Biol.* 14: 420-427.

Cahill, A. E., Aiello-Lammens, M. E., Fisher-Reid, M. C., Hua, X., Karanewsky, C. J., Ryu, H. Y. and Wiens, J. J. (2012). How does climate change cause extinction? In *Proc. R. Soc. B* (p. rspb20121890). The Royal Society.

Caviedes-Solis, I. W., Vázquez-Vega, L. F., Solano-Zavaleta, I., Pérez-Ramos, E., Rovito, S. M., Devitt, T. J. and Nieto Montes de Oca, A. (2015). Everything is not lost: recent records, rediscoveries, and range extensions of Mexican hylid frogs. *Mesoam Herpetol.* 2: 230-241.

Chaparro-Herrera, D. J., Nandini, S., Sarma, S. S. S. and Zambrano, L. (2011). Feeding behaviour of larval *Ambystoma mexicanum*. *Amphibia-Reptilia.* 32: 509-517.

Chaparro-Herrera, D. J., Nandini, S. and Sarma, S. S. S. (2013). Effect of water quality on the feeding ecology of axolotl *Ambystoma mexicanum*. J. *Limnol*. 72: 555-563

Costa, Z. J. and Vonesh, J. R. (2013). Interspecific differences in the direct and indirect effects of two Neotropical hylid tadpoles on primary producers and zooplankton. *Biotropica*. 45: 503-510.

Croteau, M. C., Davidson, M. A., Lean, D. R. S. and Trudeau, V. L. (2008). Global increases in ultraviolet B radiation: potential impacts on amphibian development and metamorphosis. *Physiol. Biochem. Zool.*, 81: 743-761.

Crump, D. (2001). The effects of UV-B radiation and endocrine-disrupting chemicals (EDCs) on the biology of amphibians. *Environ*. *Rev*. 9: 61-80.

Dahms, H. U. and Lee, J. S. (2010). UV radiation in marine ectotherms: molecular effects and responses. *Aquat. Toxicol*. 97: 3-14.

Durlak, J. A. (2009). How to select, calculate, and interpret effect sizes. J Pediatr Psychol. jsp004.

Fischer, J. and Phillips, N. E. (2014). Carry-over effects of multiple stressors on benthic embryos are mediated by larval exposure to elevated UVB and temperature. *Glob. Change Biol.* 20: 2108-2116.

Frías-Alvarez, P., Zúñiga-Vega, J. and Parra-Olea, G. (2010a). UV-B radiation severely affects embryo development in the Mexican axolotl. *Anim. Biol*. 60: 299–318.

Frías-Alvarez, P., Zúniga-Vega, J. J. and Flores-Villela, O. (2010b). A general assessment of the conservation status and decline trends of Mexican amphibians. *Biodivers Conserv.* 19: 3699-3742.

Galloy, V. and Denoël, M. (2010). Detrimental effect of temperature increase on the fitness of an amphibian *(Lissotriton helveticus*). *Acta Oecol*. 36: 179-183.

Garcia, T. S., Romansic, J. M.and Blaustein, A. R. (2006). Survival of three species of anuran metamorphs exposed to UV-B radiation and the pathogenic fungus *Batrachochytrium dendrobatidis*. *Dis. Aquat. Org*. 72: 163-169.

Gualandris-Parisot, L., Husson, D., Bautz, A., Durand, D., Kan, P., Aimar, C. and Dournon, C. (2002). Effects of space environment on embryonic growth up to hatching of salamander eggs fertilized and developed during orbital flights. *Biol. Sci. Space.* 16: 3-11.

Gunderson, A. R., Armstrong, E. J. and Stillman, J. H. (2016). Multiple stressors in a changing world: the need for an improved perspective on physiological responses to the dynamic marine environment. *Ann Rev Mar Sci.* 8: 357-378.

Häder, D. P., Helbling, E. W., Williamson, C. E. and Worrest, R. C. (2011): Effects of UV radiation on aquatic ecosystems and interactions with climate change. *Photochem. Photobiol. Sci.* 10: 242-260.

Häder, D. P. (2012). UV-B Impact on the Life of Aquatic. *Modern Trends in Applied Aquatic Ecology,* 149-172.

Hansen, A. J., DeFries, R. S. and Turner, W. (2012). Land use change and biodiversity. In *Land Change Science* (pp. 277-299). Springer Netherlands.

Harper, E. B., Patrick, D. A. and Gibbs, J. P. (2015). Impact of forestry practices at a landscape scale on the dynamics of amphibian populations. *Ecological Applications*, 25: 2271-2284.

Hinman, A. (2016). Analysis of Global Gene Expression and Notch Pathway Activity During Tastebud Development in *Ambystoma mexicanum*.

Hölting, M., Bovolo, C. I. and Ernst, R. (2016). Facing Complexity in Tropical Conservation: How Reduced Impact Logging and Climatic Extremes Affect Beta Diversity in Tropical Amphibian Assemblages. *Biotropica*.

Hussain, Q. A. (2012). Global amphibian declines: a review. *Int. J. Biodivers. Conserv.* 4: 348-357.

IUCN. (2009). Red List of Threatened Species, version 2009.1. *ee www.iucnredlist.org* (date last accessed 1 May 2009).

Kaufman, L. (1992). Catastrophic change in species-rich freshwater ecosystems. *BioScience*. 42: 846-858.

Kern, P., Cramp, R. L. and Franklin, C. E. (2014). Temperature and UV-B-insensitive performance in tadpoles of the ornate burrowing frog: an ephemeral pond specialist. *J. Exp. Biol.* 217: 1246-1252.

Mačát, Z., Jeřábková, L., Reiter, A., Rulík, M. and Jablonski, D. (2015). Malformations and body injuries in a hybrid zone of crested newts (Caudata: Salamandridae: *Triturus cristatus* superspecies). *Acta Herpetológica*, *10*(2), 135-141.

Marquis, O., Millery, A., Guittonneau, S. and Miaud, C. (2006). Toxicity of PAHs and jelly protection of eggs in the common frog *Rana temporaria*. *Amphibia-Reptilia*. 27: 472-475.

Marquis, O. and Miaud, C. (2008). Variation in UV sensitivity among common frog *Rana temporaria* populations along an altitudinal gradient. *Zool*. 111: 309-317.

Měráková, E. and Gvoždík, L. (2009). Thermal acclimation of swimming performance in newt larvae: the influence of diel temperature fluctuations during embryogenesis. *Funct. Ecol*. 23: 989-995.

Mills, N. E. and Ward, Z. A. (2015). Egg Hypoxia Decreases Posthatching Survival and Delays Metamorphosis in *Ambystoma maculatum* (Spotted Salamander). *J. Herpetol*. 49: 616-620.

Muir, A. P., Biek, R., Thomas, R. and Mable, B. K. (2014). Local adaptation with high gene flow: temperature parameters drive adaptation to altitude in the common frog (*Rana temporaria*). *Mol. Ecol*. 23: 561-574.

Ochoa-Ochoa, L. M., Rodríguez, P., Mora, F., Flores-Villela, O. and Whittaker, R. J. (2012). Climate change and amphibian diversity patterns in Mexico. *Biol. Conserv.* 150: 94-102.

Oromi, N., Camarasa, S., Sanuy, I. and Sanuy, D. (2015). Variation of growth rate and survival in embryos and larvae of *Rana temporaria* populations from the Pyrenees. *Acta Herpetológica*, 10(2), 85-91.

Ovaska, K., Davis, T. M. and Flamarique, I. N. (1997). Hatching success and larval survival of the frogs *Hyla regilla* and *Rana aurora* under ambient and artificially enhanced solar ultraviolet radiation. *Can. J. Zool.* 75: 1081-1088.

Palmer, M. A., Lettenmaier, D. P., Poff, N. L., Postel, S. L., Richter, B. and Warner, R. (2009). Climate change and river ecosystems: protection and adaptation options. *Environ. Manage*. 44: 1053-1068.

Park, D., McGuire, J. M., Majchrzak, A.L., Ziobro, J. M. and Eisthen, H. L. (2004). Discrimination of conspecific sex and reproductive condition using chemical cues in axolotls (*Ambystoma mexicanum*). *J. Comp Physiol A*. 190: 415-427.

Parmesan, C. and Matthews, J. (2006). *Biological impacts of climate change. Principles of Conservation Biology*. Sunderland, Massachusetts: Sinauer Associates, 333-374.

Parra-Olea, G., Martínez-Meyer, E. and De León, G. P. P. (2005). Forecasting Climate Change Effects on Salamander Distribution in the Highlands of Central Mexico. *Biotropica*. 37: 202-208.

Pilliod, D. S., Muths, E., Scherer, R. D., Bartelt, P. E., Corn, P. S., Hossack, B. R. and Gaughan, C. (2010). Effects of amphibian chytrid fungus on individual survival probability in wild boreal toads. *Conserv. Biol.* 24: 1259-1267.

Quintero, E., Thessen, A. E., Arias-Caballero, P. and Ayala-Orozco, B. (2014): A statistical assessment of population trends for data deficient Mexican amphibians. *Peer. J, 2*, e703.

Recuero, E., Cruzado-Cortes, J., Parra-Olea, G. and Zamudio, K. R. (2010). Urban aquatic habitats and conservation of highly endangered species: the case of *Ambystoma mexicanum* (Caudata, Ambystomatidae). In *Annales Zoologici Fennici* (pp. 223-238). Finnish Zoological and Botanical Publishing Board.

Ripple, W. J., Estes, J. A., Beschta, R. L., Wilmers, C. C., Ritchie, E. G., Hebblewhite, M. and Schmitz, O. J. (2014). Status and ecological effects of the world's largest carnivores. *Science*. 343: 1241484.

Rollins-Smith, L. A. (2009). The role of amphibian antimicrobial peptides in protection of amphibians from pathogens linked to global amphibian declines. Biochim. Biophys. *Acta-Biomembranes*. 1788: 1593-1599.

Roth, T. L. and Obringer, A. R. (2003). Reproductive research and the worldwide amphibian extinction crisis. *Conservation Biology Series-Cambridge*. 359-374.

Shoots, J., Fraccalvieri, D., Franks, D. G., Denison, M. S., Hahn, M. E., Bonati, L., and Powell, W. H. (2015). An Aryl Hydrocarbon Receptor from the Salamander *Ambystoma mexicanum* Exhibits Low Sensitivity to 2, 3, 7, 8-Tetrachlorodibenzo-p-dioxin. *Environ. Sci. Technol.* 49: 6993-7001.

Shu, L., Suter, M. J. F., Laurila, A. and Räsänen, K. (2015). Mechanistic basis of adaptive maternal effects: egg jelly water balance mediates embryonic adaptation to acidity in *Rana arvalis*. *Oecol*. 179: 617-628.

Smith, G. D., Hopkins, G. R., Mohammadi, S., Skinner, H. M., Hansen, T., Brodie, E. D. and French, S. S. (2015). Effects of temperature on embryonic and early larval growth and development in the rough-skinned newt (*Taricha granulosa*). *J. Therm Biol.* 51: 89-95.

Sokal, R. R. and Rohlf, F. J. (2000). *Biometry*. San Francisco: W.H. Freeman.

Tapley, B., Rendle, M., Baines, F. M., Goetz, M., Bradfield, K. S., Rood, D. and Routh, A. (2015). Meeting ultraviolet B radiation requirements of amphibians in captivity: A case study with mountain chicken frogs (*Leptodactylus fallax*) and general recommendations for preǧrelease health screening. *Zoo Biol.* 34: 46-52.

Tarvin, R. D., Silva Bermúdez, C., Briggs, V. S. and Warkentin, K. M. (2015). Carry-over effects of size at metamorphosis in red-eyed treefrogs: higher survival but slower growth of larger metamorphs. *Biotropica*. 47: 218-226.

Thurman, L. L., Garcia, T. S. and Hoffman, P. D. (2014). Elevational differences in trait response to UV-B radiation by long-toed salamander populations. *Oecol*. 175: 835-845.

Tomanek, L. (2010). Variation in the heat shock response and its implication for predicting the effect of global climate change on species' biogeographical distribution ranges and metabolic costs. *J. Exp. Biol.* 213: 971-979.

Tweedell, K. S. (2013). In Oocytes and Embryos of *Rama pipiens*. *Biology of Amphibian Tumors,* 229.

Williamson, C. E, Zepp, R. G., Lucas, R. M., Madronich, S., Austin, A. T., Ballaré, C. L. and Robinson, S. A. (2014). Solar ultraviolet radiation in a changing climate. *Nat. Clim. Change.* 4: 434-441.

Wong, C. Y., Teoh, M. L., Phang, S. M., Lim, P. E. and Beardall, J. (2015). Interactive effects of temperature and UV radiation on photosynthesis of *Chlorella* strains from polar, temperate and tropical environments: differential impacts on damage and repair. *PloS One*, 10 (10), e0139469.

Woods, H. A. (1999). Egg-mass size and cell size: effects of temperature on oxygen distribution. *Amer. Zool.* 39: 244-252.

Yu, S., Weir, S. M., Cobb, G. P. and Maul, J. D. (2014). The effects of pesticide exposure on ultraviolet-B radiation avoidance behavior in tadpoles. *Sci. Total Environ.* 481: 75-80.