Effectiveness of Frog Skin Secretions Against UV-B Radiation

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Abstract

As an increasing number of their species face possible extinction, amphibian populations have been steadily declining over the past decades (IUCN). While various factors have been suggested or proven to be partially responsible for this reduction, increased ultraviolet B radiation (UVBR) appears to be one of the more prominent explanations, especially when considering the continual thinning of the Earth's ozone layer due to anthropogenic climate change. However, it has also been discovered that skin secretions, which are reported to function as a component of amphibians' innate immune systems, may have an effect on UVBR (Cramp and Franklin). With this in mind, we wondered if the skin secretions of two local frog species, California tree frogs (*Pseudacris cadaverina*) and Pacific tree frogs (*Pseudacris regilla*), might be indicative of this. We hypothesized that a small glass slide consisting of an individual frog's secretory layer would have a lower ultraviolet radiation reading than that of an unaltered glass slide, suggesting that a frog's secretions protect it from a quantifiable amount of harmful UVBR. Using a consistently positioned UV meter, six frogs of each species were collected and tested independently at the streambank in Arroyo Sequit Park. Wavelengths of an individual's secretions were recorded five separate times, with control measurements being taken before and after each trial. While nearly all secretions from both sampled species saw a slight decrease in UVBR levels when compared to the control readings, our findings were not significant enough to support our hypothesis, possibly suggesting that amphibians' skin secretions act as only a minimal deterrent to ultraviolet radiation.

Introduction

Amphibian population decline has become a ubiquitous concern in discussions of ecosystem health and global biodiversity. While many other endangered vertebrate species are threatened by nearby anthropogenic presence, amphibians face community decreases in habitats that have been left mostly untouched, insinuating that the cause of this widespread and rapid reduction may be both more complicated and extensive than was originally believed (Alton et al.). As stratospheric ozone continues to allow more ultraviolet radiation to enter the atmosphere, it would appear that human-induced climate change might be accountable for these increased amphibian mortalities (Novales Flamarique et al.).

Despite this, it has been reported that some species have defenses against UVBR, allowing them to limit or repair the damage after exposure (Blaustein and Belden).Certain research has discovered the existence of embryonic protection and repair when amphibian eggs are exposed to harmful ultraviolet rays, which is indicative of potential differences in UV sensitivity among species (Blaustein et al.). Levels and activity of photolyase were examined among developing larvae at natural oviposition areas, and it was found that interspecies hatching success varied despite being exposed to similar levels of radiation (Blaustein et al.). These results suggest different amphibians have distinct responses to UVBR, possibly alluding to natural species-specific adaptations that have formed in consideration of continued subjection to ultraviolet rays.

With this in mind, we specifically chose to conduct our experiment at Arroyo Sequit Park, due to both the dense tree frog populations inhabiting the site and our own personal experience at the stream. During the summer of 2023, we helped conduct USGS surveys as a part of Dr. Kats' behavioral ecology laboratory at multiple wetland communities, including the creek at Arroyo Sequit Park. Due in part to Southern California's recent wildfires, the habitat lacks much vegetative cover, making indigenous amphibian populations susceptible to high UVB exposure.

Methods

A Solar Light (Model PMA2100) UV meter was placed upon a rock on the streambank in Arroyo Sequit Park in an area that had a high volume of both California and Pacific tree frogs. Reflective black tape was used to cover the meter's surface, leaving only a small circular slit exposed and ensuring that only that area would be measured. A circular microscope coverslip was then set upon the opening; this functioned as our arrangement for our control readings. Using nitrile examination gloves, a frog from one species was dipped into the water and carefully held near the meter. The frog's dorsal side was gently swabbed with a sterile cotton-tipped applicator in order to extract the secretion from the individual's epidermal layer, then the film from the secretion was rubbed onto a different coverslip. After replacing the original glass coverslip with the one that had the secretion on it, we recorded the UV wavelength data reported by the meter. A second control reading was taken following the removal and cleaning of the used coverslip. This procedure was repeated another two times for six frogs of each species, meaning that each individual underwent three consecutive trials before being released.

Our collected data was then organized into three categories: the UV reading (ppm) before the skin secretion was applied to the meter, the UV reading with the secretion applied, and the UV reading after the meter had been wiped clean. An average of the data points preceding and following the skin secretion was taken in order to create a single control value for the values belonging to each frog. Unfortunately during our outing, we failed to obtain a control value for another glass slide that the secretion was applied to and then placed over the UV meter, creating a major discrepancy. To resolve this issue, we performed a separate experiment attempting to find the amount of radiation that is mitigated by only a singular glass slide. To make these trials correspond with the original ones, we converted the newly acquired data into a percentage. This percentage was then multiplied to every experimental value and later added back to properly account for the rays that a single glass slide mitigates, ensuring that our control values had been correctly adjusted to make up for our error. Once this was rectified, two separate, standard unpaired T-tests were utilized to determine if there was a significant difference between the control values and each frog species' secretory UV readings.

Results

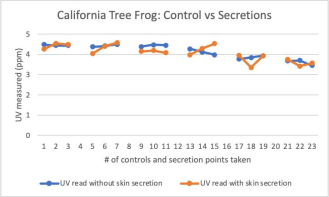

Figure 1: The following graph shows the differences in ppm between the UV reading with the skin secretion applied (orange) and the UV reading without skin secretion applied (blue) for the Cadaverina tree frogs. The connected lines represent individual frogs while the dots represent individual data points. It can be observed that there is not a significant difference between the control and the experimental values.

Table 1: Data from the unpaired, two-tailed t-test comparing California tree frogs to control readings.

t	df	p
- 0.1	44	0.920799

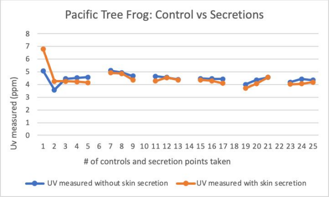

Figure 2: The following graph shows the differences in ppm between the UV reading with the skin secretion applied (orange) and the UV reading without skin secretion applied (blue) for the Pacific tree frogs. The connected lines represent individual frogs while the dots represent individual data points. It can be observed that there is not a significant difference between the control and the experimental values.

Table 2: Data from the unpaired, two-tailed t-test comparing Pacific tree frogs to control readings.

t	df	p
0.1	48	0.920761

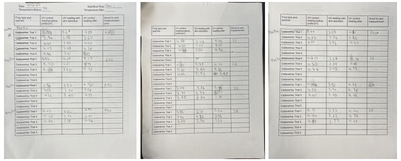

Figure 3: The following images are the three original datasheets used in the field. The headings read as follows: frog species, snout to vent measurement, UV control reading before, UV reading with frog skin secretion, and UV control reading after the secretion was collected. The data was then transcribed to a Microsoft Excel spreadsheet for statistical analyses.

Discussion

The primary objective of this experiment was to test whether the skin secretions of California and Pacific tree frogs deflect UV-B radiation from the sun. By testing the readings from a UV meter with and without the epidermal layer applied to it, we were able to gather two sets of control and experimental values: one for *Pseudacris cadaverina* and the other for *Pseudacris regilla*. We used an unpaired, two-tailed t-test to see if there was any significant difference between these control and experimental values. Our significance was set at $\alpha = 0.05$, and our null hypothesis assumed there would be no significant difference between the frogs' secretion readings and the control readings. The p-value we obtained for the Pacific tree frogs and Cadaverina tree frogs were $p = 0.920761$ and $p = 0.920799$, respectively, meaning that we could not reject the null hypothesis for either species. A peripheral aspect of this experiment entailed a comparison between the two frog species. After comparing our data, we immediately noticed similarities between the two species' p-values. Due to this resemblance, we were led to hypothesize that location may play a factor in skin secretion effectiveness, especially considering how each frog was taken from the same area (Arroyo Sequit Park).

While it is certainly likely that the insignificance of our data was solely because of the frogs' morphologies, it is possible that the sources of error in our experimental design could have also been a contributing factor. As was previously stated in an earlier section, we failed to take a control reading of a second glass slide that was stacked upon the first layer on the meter, resulting in the need to run another subset of trials on a later date to account for the mistake. Differences in temperature, cloud cover, and UV index between the two data collection days might have played a part in our statistical results. Moreover, each frog was consistently dipped back into the stream in order to replenish their epidermal layer, introducing a variable in the form of the flowing water. While probably trivial, the lack of uniformity of current may have influenced the next secretion sampling, leading to a potentially unnatural deviation being reported. While difficult to know, these inconsistencies could have skewed the results we derived from our data and contributed to the observed outcome.

To conclude, we sought to discover whether or not the skin secretions of two local frog species, California tree frogs (*Pseudacris cadaverina*) and Pacific tree frogs (*Pseudacris regilla*), would successfully mitigate harmful ultraviolet-B radiation. Despite the insignificance of our results, this subject warrants further investigation due to amphibian responses to UVBR remaining relatively unknown. Furthermore, continued anthropogenic climate change has made studying the effects of these harmful rays even more crucial, especially when considering how ozone depletion is impacting not only amphibians, but all biodiversity. In the future, we would appreciate the opportunity to resume examining the benefits of local amphibian species skin secretions in relation to ultraviolet rays, with particular focus on improving our experimental design to avoid the sources of error we previously mentioned. For example, trials that compared an individual's UV readings before and after the epidermal coating was removed could be worth conducting, in light of a possible decrease in the protection against radiation that the secretions might provide. It may also be worth testing the same two species at different stream sites; because the statistics were quite similar among both frog types, location could play a role in either the amount or effectiveness of the secretion, possibly indicating that adaptation to an area's ultraviolet radiation exposure is occurring across different amphibian habitats. There is an urgent need to bring declining biodiversity to a halt, and one of the most promising ways of accomplishing that is additional research, especially in fields that pertain to human-induced reductions such as this one.

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